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TUMOURS OF THE EPIPHYSIS CEREBRI

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(PLATES I-VI)

THE comprehensive study of Gladstone and Wakeley (1940), confirms the view long held by embryologists, that the human pineal organ is but a vestigial remnant of paired median eyes. Thought by Descartes to be the seat of the soul, it subsequently had ascribed to it endocrine glandular functions, and various unconvincing experimental data were produced in support of this theory. However, endocrine disturbances formerly ascribed to secretory functions of pineal neoplasms have all been produced by other lesions involving the hypothalamus ; and for lack of evidence to the contrary, the pineal organ must still be classed as a vestigial structure.

The symptoms which may be produced by neoplasms of the pineal region can be listed under four groups :—(i) Those due to increased intracranial pressure from blockage of the Sylvian aqueduct. (ii) Disturbance of hypothalamic function by growth into the third ventricle, producing abnormalities of sexual and skeletal development, obesity, diabetes insipidus and hypersomnia. (iii) Collicular symptoms due to mid-brain damage. (iv) Cerebellar disturbances caused by interference with cerebellar connections in the mid-brain, and also by growth of the neoplasm backwards beneath the tentorium cerebelli with consequent compression or invasion of the cerebellum.

Dorothy Russell (1944) has shown that the type of tumour hitherto ;garded as a typical " pinealoma " is probably an atypical teratoma. ·In her opinion, true pineal neoplasms are extremely rare.

Of 236 " pineal " tumours recorded up to the end of 1946 fewer than one-fifth are true epiphyseal tumours. The remainder are the

atypical teratomas described by Dorothy Russell, other more obvious teratomas including chorionepithelioma, glial tumours, hypertrophies and cysts.

The object of this paper is to present some of the histological characteristics of true pineal neoplasms additional to the " spongioblastic pinealoma " of Horrax and Bailey (1925), and also to adduce further evidence of the teratoid nature of most of the so-called " pinealomas."

<div align="center">CASE REPORTS</div>

<div align="center">Case 1</div>

The patient, a boy aged 11 years, was admitted to the department of neurosurgery, Royal Prince Alfred Hospital, on 4th May 1939, in the care of Mr R. Money. He was an Italian boy who had been in Australia only 15 months. For two years he had suffered from intermittent headaches, weakness of the limbs, staggering gait, gradual enlargement of the head and frequent vomiting attacks.

On examination, there was generalised enlargement of the skull, more marked in the occipital region. The fundi showed bilateral secondary optic atrophy, although the visual acuity on each side was 6/12. Bilateral horizontal nystagmus was present, and there was a stamping ataxic gait on a wide base. All tendon reflexes were hyperactive.

Blood Wassermann and Kline reactions and the Casoni test were negative. Stereoscopic radiography of the skull showed flattening of the sella turcica, with erosion of the posterior and to a less extent of the anterior clinoid processes. Lumbar puncture produced clear cerebrospinal fluid at a pressure of 300 mm. of water ; it contained only a trace of globulin. Ventricular puncture disclosed bilateral communicating hydrocephalus, confirmed by the introduction of methylene blue.

Considering that the lesion was a mid-line cerebellar tumour, sub-occipital craniotomy under nitrous oxide-oxygen-ether anæsthesia was carried out on 9th May 1939. No tumour was found in the posterior cranial fossa, the only abnormality being a few adhesions around the fourth ventricle foramina. The patient's condition gradually deteriorated following operation, and he died on the fourth post-operative day after lapsing into coma. A full post-mortem examination was carried out the same day.

Autopsy. The cranium was enlarged, with " thumbing " impressions on the inner table. The upper part of the sella turcica was enlarged as a result of contact with the ballooned floor of the third ventricle. The dorsum sellæ was almost obliterated and the pituitary body was flattened. The dilated third ventricle bulged in front of and behind the optic chiasma, which embraced its lower portions. In the upper and anterior portion of the cerebellum was a large thin-walled cyst.

Median sagittal section of the brain revealed great dilatation of the third and lateral ventricles and destruction of the septum lucidum. In the Sylvian aqueduct lay a tongue of tumour tissue which originated from a large neoplasm occupying the whole of the posterior portion of the mid-brain above the aqueduct. Posterior to this portion of the tumour there was a cystic extension which projected beneath the tentorium cerebelli, compressing and displacing the anterior lobe of the cerebellum without actually invading it. The cyst was thin walled and contained blood-stained fluid. The tumour (including the cystic portion) measured 4·5 cm. antero-posteriorly and 2·2 cm. in its vertical axis (fig. 1).

Microscopic appearances (fig. 2). The tumour is very cellular, with little

PLATE I

PINEAL TUMOURS

Fig. 1.—Case 1. Mid-sagittal section of brain showing a cystic tumour compressing the cerebellum and a tongue of tumour tissue growing down the cerebral aqueduct. The white body above the tumour is a portion of the splenium of the corpus callosum. Note the greatly dilated third and lateral ventricles.

stroma and few vessels. The cells are small and contain spherical nuclei. Many of them have scanty cytoplasm, which in some cases is drawn out into stubby tails and in others into a short argyrophilic process. There is no obvious pattern but the appearances resemble those of a neuroblastoma. This tumour is very invasive and portions of tissue taken from near its periphery contain neurones of the mid-brain nuclei in a fairly good state of preservation.

Case 2

The patient, a female aged 19 years, was admitted to the department of neurosurgery, Royal Prince Alfred Hospital, on 17th March 1942, in the care of Mr G. Phillips. Eight months previously she had developed occipital and nuchal pain, followed by episodic vomiting, diplopia, attacks of giddiness and unsteadiness of gait. More recently there had been impairment of vision, gradually increasing deafness and coarse tremor in the hands.

On examination, there was right-sided papilloedema and the pupils did not react to light. Horizontal nystagmus was present on looking to the right. There was intention tremor in the left arm, with lateral flexion of the head to the left and a tendency to fall backwards and to the left during Romberg's test. Both knee jerks were hyperactive and the tendon reflexes in the left arm were exaggerated.

Plain radiography of the skull revealed some diaschisis of the sagittal suture, with " thumbing " in the frontal regions. The sella turcica was normal in size but its floor was somewhat uneven. Lumbar puncture disclosed clear fluid at a pressure of 230 mm. of water, with normal constituents. Ventriculo-graphy on 26th March 1942, disclosed symmetrical dilatation of the lateral ventricles and dilatation of the third ventricle. No air could be made to enter the Sylvian aqueduct.

In the belief that a cerebellar tumour was present, sub-occipital craniotomy was performed on 1st April 1942, under rectal Avertin and local anaesthesia. No tumour was found and the wound was closed. The patient recovered satisfactorily and was discharged " improved " on 14th April 1942.

She was re-admitted on 30th June 1942 because of a recrudescence of symptoms, and a second operation was carried out on 8th July through the previous decompression. Again no tumour was found in the cerebellum, so the vermis was split to disclose the floor of the fourth ventricle. A catheter was then introduced into the Sylvian aqueduct, but could only be passed a short distance before an obstruction was encountered. The patient's condition at this stage was poor, so the wound was closed, the dura mater being left open. The patient died that afternoon and a full post-mortem examination was carried out the same day.

Autopsy. The surface of the brain was congested, the cerebral hemispheres were enlarged and the gyri flattened. Median sagittal section after fixation revealed a tumour in the third ventricle, extending backwards beneath the splenium of the corpus callosum to compress and invade the cerebellum. It measured 5·0 cm. from before backwards and 2·2 cm. from above downwards. It was attached to the posterior part of the lateral walls of the third ventricle and to the deep surface of the quadrigeminal plate and pineal region. It projected backwards to compress the superior surface of the cerebellum. In appearance it was pinkish-white, and it was soft and friable to the touch. In front of the tumour there were small implantations of tumour tissue in the lateral walls of the third ventricle, the largest being in the massa intermedia on the right side. There was no evidence of a pineal body. The thymus was enlarged, weighing 150 g., and there was a cystic adenoma of the thyroid.

Microscopic appearances (fig. 3). The tumour is very cellular and contains relatively few blood vessels, most of which are very small and are accompanied

by scanty connective tissue. Tumour cells are orientated about the vessels in the manner of an ependymoma. However, the main mass of tumour cells is not related to vessels and in the non-vascular areas there are many small pseudo-rosettes. The cells are small, with rounded nuclei which contain a coarsely granular chromatin network. Nucleoli are present in some nuclei. The scanty cytoplasm is drawn out into a short pointed tail at one pole, so that the individual cell is shaped like a short thick carrot. The cells arranged around vessels have longer and finer processes which radiate centrally. These processes are deeply impregnated by silver methods (fig. 4). The neoplastic plaques in the lateral walls of the third ventricle have a similar histological appearance, with normal ependyma at the periphery. No calcification can be detected. The pituitary gland is normal.

Case 3

The patient, a male aged 47 years, was admitted to the department of neurosurgery, Royal Prince Alfred Hospital, on 9th April 1944, in the care of Mr G. Phillips. Eight years prior to admission he developed episodic frontal headaches accompanied by nausea and vomiting. He also noticed deterioration of vision and for a week suffered from diplopia. This phase lasted about one month. Over the next few years he gradually became more and more tired, his gait became unsteady and he had attacks of headache, vomiting and severe ataxia about every three months. In the two years before admission he became unconscious during these attacks and finally became unable to walk. His hearing gradually deteriorated, diplopia returned and his memory became much worse. For six months he had had tingling in his right hand. In the last three or four months he had lost all libido ; his wife had had one child four months before his admission.

On examination he was found to be stuporose and somewhat uncooperative. Visual acuity was poor in both eyes but the visual fields were full. The margins of both optic papillæ were blurred but the physiological cups were still present. There was paresis of the right lateral rectus muscle and bilateral nerve deafness. The remainder of the neurological examination disclosed no abnormality except bilateral ankle clonus. Blood pressure was 150/100 mm. Hg.

Plain radiography of the skull disclosed some deepening of the sella turcica and an extensive area of calcification in the region of the pineal body. Lumbar puncture produced clear cerebrospinal fluid under a pressure of 250 mm. of water, with normal constituents. Ventriculography on 19th April 1944 showed considerable enlargement of the lateral and third ventricles, but no air could be induced to enter the Sylvian aqueduct.

In the belief that the lesion was a cerebellar tumour, sub-occipital craniotomy was performed on 3rd May 1944 under rectal Avertin and local anæsthesia. No tumour could be found on exploration with a brain needle, so the wound was closed, as it was considered that an inoperable mid-brain tumour was present. The patient died eight days later from respiratory failure and a full post-mortem examination was carried out the same day.

Autopsy. There was marked flattening of the gyri, enlargement of the cerebral hemispheres and gross venous congestion. Median sagittal section of the brain disclosed a tumour in the third ventricle (fig. 5). It arose from the quadrigeminal region, occupied most of the third ventricle and extended posteriorly beneath the splenium of the corpus callosum to lie in contact with the cerebellum. The tumour was encapsulated, reddish-brown in colour and measured 3·5 cm. antero-posteriorly and 2·8 cm. in its vertical axis. It obstructed the ventricular system in the third ventricle, so that there was marked dilatation of the lateral ventricles. No pineal body could be found.

Microscopic appearances. The tissue is moderately cellular (fig. 6) and the majority of cells are fairly uniform in type, though there are occasional giant

FIG. 2.—Case 1. Microscopic appearance of this undifferentiated pinealoblastoma. Hæmatoxylin and eosin. ×180.
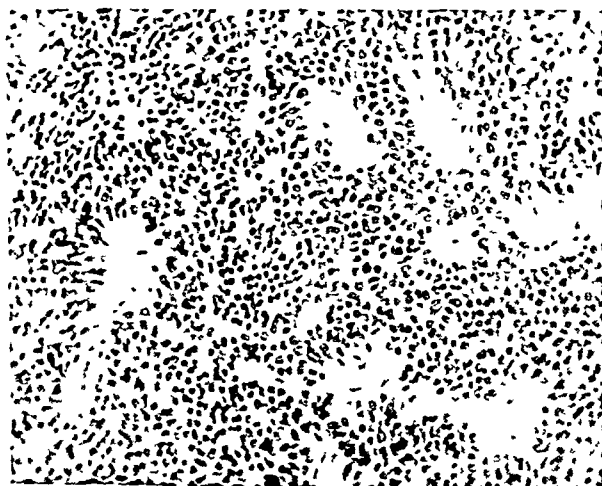
FIG. 3 —Case 2. Pinealoblastoma. Microscopic appearance of the tumour. Hæmatoxylin and eosin. ×180.
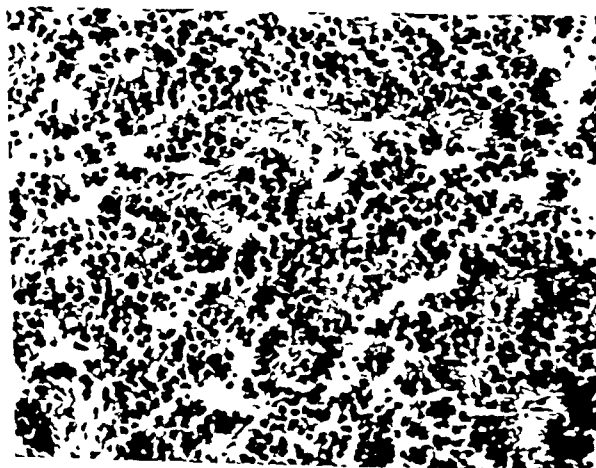
FIG. 4 —Case 2. Silver preparation to show the cell processes Roger's method ×180.

cells with one or more irregular hyperchromatic nuclei. There is a definite pattern throughout, in which the cells are arranged partly in irregular cords and partly around small patches of granular matrix which may or may not contain a vascular connective-tissue stroma. The central stroma is connected to adjacent areas by argyrophil reticulin bundles. In these central areas where no connective tissue is demonstrable, a few microglial and oligodendroglial cells can be demonstrated by del Rio-Hortega's silver carbonate methods.

The tumour cells have small round or ovoid nuclei and scanty cytoplasm which forms a tail. In others this cytoplasmic process is longer, and either bifurcates or in a few instances divides into several short thin arborisations. There are also occasional cells in which there is a process at one pole which terminates in claw-like filaments, while at the other the cytoplasm opens out into a flattened expansion from which cilia can be seen to arise.

Case 4

The patient, a male baby aged 20 months, was admitted to the Royal Prince Alfred Hospital on 8th January 1947, in the care of Mr R. Money. Symptoms were first noticed 3 months before admission. At that time the mother noticed that the child kept his rather large head tilted to the left. Two weeks later he began to vomit, and one week later it was found that the right eye would not turn to the left and that there was marked dilatation of the pupils. One month before admission right facial paresis was present and the child became drowsy and irritable.

On examination the child was found to be very drowsy. The head was large, measuring 21 inches in circumference. There was some rigidity of the neck and the muscles generally were hypotonic. There was marked bilateral papillœdema, with hæmorrhages in the peri-macular region, and a spontaneous horizontal nystagmus was present, with the quick component towards the right. All tendon reflexes were absent except the left biceps jerk ; a left Babinski response was elicited.

A blood count showed a mild anæmia. Plain radiography of the skull revealed no abnormality. Ventricular estimation, performed on 11th January 1947, revealed bilaterally dilated lateral ventricles. Introduction of air into the left ventricle showed no air entering the right. When this ventricle was filled directly, bilaterally symmetrical hydrocephalus was demonstrated, together with dilatation of the anterior portion of the third ventricle. A filling defect was seen in the posterior portion of the third ventricle.

With a presumptive diagnosis of third ventricular tumour, a left parieto-occipital craniotomy was performed on 13th January 1947 under local anæsthesia. An opening was made through the parietal lobe into the left lateral ventricle. The third ventricle was opened through its roof, but bleeding obscured the operative field and no tumour could be seen. The wound was closed and the patient returned to bed, dying 12 hours later. A cranial examination was carried out *post mortem* the following day.

Autopsy. The right side of the brain and cerebellum was bigger than the left. On removing the cerebellum, a tumour mass could be seen deep in the cerebro-cerebellar sulcus. It extended backwards under and into the anterior lobe of the cerebellum, compressing the upper end of the fourth ventricle. Horizontal section of both brain and cerebellum disclosed, in the latter, tumour masses in both hemispheres fanning out from a central mass. On the left side, a second tumour mass, 3 × 2·5 cm., lay in the pia mater and was pressing deeply into the left cerebellar hemisphere. The ventricular system was dilated above the Sylvian aqueduct. Superiorly, the tumour tissue compressed the quadrigeminal plate from the dorsal aspect, occluding the Sylvian aqueduct. It also extended forwards and downwards into the posterior portion of the third

ventricle. At the rostral end of the tumour, a pedunculated mass was present which measured 1·3 cm. in diameter. Its gross structure was similar to that of the main mass.

Microscopic appearances. A general survey of the tumour tissue reveals a characteristic pattern (fig. 7). Dense argyrophil connective tissue divides the tumour into lobules (fig. 8). This connective tissue is not obvious with ordinary connective-tissue stains. There are also groups of tumour cells within the meshes of the reticulin network. The cells are small, with spherical or ovoid nuclei in which a dense chromatin network can be seen, and in some cases there is a nucleolus. For the most part there is little cytoplasm. In the larger lobular masses the cytoplasm of most cells is pulled out into a small polar process. This is a more prominent feature in the smaller groups of cells within the meshes of the connective tissue. The vessels, which are not numerous, are situated within the stromal walls of the lobules.

COMMENT ON CASES 1-4

On histogenetic grounds, case 1 represents a tumour of the primitive anlage without much attempt at differentiation. Case 2 is similar in structure to the fœtal pineal body in that there are cords of connective tissue around which the cells are orientated as in the 180 mm. embryo (figs. 10 and 11). Case 3 shows little differentiation towards a lobular structure but there is an attempt at cellular differentiation and the picture resembles that of a neuroblastoma. In case 4 the appearances are those of the pinealoblastoma described by del Río-Hortega (1933). He found similar differentiating cells in relation to the stroma. This tumour is apparently a further stage of differentiation of the " mosaic " type of pineal tumour ; instead of lobules of large cells being surrounded by smaller cells, they are now surrounded by connective tissue. Further differentiation of cells towards the normal pineal parenchymal cells results in the pinealocytoma, as described and illustrated by del Río-Hortega.

Case 5

This patient, a male aged 18 years, was admitted to hospital in the care of Mr G. E. Phillips on 30th November 1944. He was found to have bilateral ocular palsies, deafness of central type and marked papillœdema. He was of a lethargic and dull disposition. No other details of history or clinical examination are available.

Plain X-ray examination demonstrated displacement of a large calcified pineal body anteriorly and inferiorly. Ventriculography showed dilatation of the third and lateral ventricles. The aqueduct of Sylvius could be seen distorted below a tumour which extended forward into the posterior part of the third ventricle from the roof of the mid-brain. A diagnosis of a large tumour arising from the pineal region was made. Craniotomy was performed on 5th December 1944. The tumour was found to be inoperable. The condition of the patient deteriorated and he died on 22nd December 1944.

Autopsy. (Cranial examination only.) Sagittal section of the brain disclosed a large tumour in the mid-brain region. It appeared to arise from the posterior end of the roof of the third ventricle and extended backwards into the cerebellum and forwards into the third ventricle. It was firm in texture and reddish-brown in colour, with flecks of yellow in its substance. It measured 7 × 4·5 × 4·5 cm. and contained a calcified mass 7·5 mm. in diameter in its anterior end.

Microscopic appearances (figs. 11 and 12). The neoplasm is a teratoid growth. In the region of the calcified nodule there is a mass of spheroidal cells mixed with collections of lymphocytes and the appearance is that of the so-called "pinealoma." Elsewhere the tumour has an alveolar pattern. These alveoli contain a variable number of vacuolated spheroidal cells. Papillæ covered by these cells project into some alveoli. The cells are large and have irregular hyperchromatic nuclei. They resemble in some degree the cells of chorionic carcinoma. Examination was limited to three paraffin blocks and no other kinds of tissue were discovered.

DISCUSSION

In this study, a number of glial tumours were discarded from the series for the reason that glial tumours originating in the epiphysis are no different from glial tumours arising elsewhere in the brain. Furthermore, as there is no distinctive pattern of glial tumours in this region, a destructive glioma may have arisen from any adjacent part of the mid-brain region, the exact site of origin being impossible to identify.

Tumours of the pineal region are predominantly teratoid. Dorothy Russell produced convincing evidence as to the teratomatous nature of the so-called pinealoma. She pointed out also that the problem of ectopic "pinealoma" in the presence of a normal epiphysis is more understandable on a teratomatous hypothesis. Theoretically it is possible on histogenetic grounds for a true pineal tumour to be associated with an apparently normal epiphysis. Failure of the two branches of the primitive diverticulum to fuse and subsequent neoplasia of one branch would provide an adequate explanation. No case of the kind, however, has been reported in the literature.

The pineal organ is a vestigial structure with no known function and true tumours arising from its essential constituent cells are rare. The "mosaic" type described by Horrax and Bailey (1925) as "spongioblastic" is an example. It resembles the developing pineal body in some respects, as does the "pinealoblastic" type of del Río-Hortega, of which one example is presented here.

It is suggested that undifferentiated epiphyseal tumours, because of their resemblance to neuroblastomas elsewhere in the body, should be designated "pinealoblastomas," and that more differentiated forms which reproduce to some extent the cyto-architecture of the normal epiphysis should be referred to as "pinealocytomas." The tumour heretofore designated "pinealoma" is apparently an atypical teratoma and should be classified with the more obviously teratoid growths as suggested by Dorothy Russell.

Classification of pineal tumours

A histogenetic classification of pineal neoplasms is suggested, as follows.

1. *Pinealoblastoma* (cases 1-4 in this series). This tumour may show a varying architectural pattern depending on how closely the

architecture of the normal epiphysis is reproduced, but basically it resembles a neuroblastoma. The spongioblastic type of Horrax and Bailey (1925) belongs to this group.

2. *Pinealocytoma.* This tumour is a more differentiated variety of 1 and more nearly approximates to the structure of the normal epiphysis.

3. *Ganglioneuroma.* Just as there are ganglion cells in the normal epiphysis, originally derived from neuroblasts, so a tumour producing ganglion cells should be expected (Horrax and Bailey, 1928; Schmincke, 1929).

4. *Glioma.* Various types of glioma have been described. The pineal body, being derived from a diverticulum of brain substance and containing its various glial components, is therefore not immune from tumours of glial origin.

5. *Teratoma* (including " atypical " teratoma) (case 5). This is the commonest neoplasm of the pineal region. Its histogenesis, as elsewhere in the body, apart from the sex glands, is obscure.

Certain other conditions which, although not true neoplasms, behave in a similar manner from the symptomatic and clinical viewpoint are *hydrops cysticus*, a condition due to failure of the primitive pineal diverticulum to become obliterated (Cooper, 1932-33), and *hypertrophy of the pineal body*, in which the commonest cause of the enlargement is hyperplasia of glial or mesodermal components.

Melanoma has been described as a primary tumour of the epiphysis (Ogle, 1899), but owing to the widespread dissemination of such tumours, the exact site of the primary growth is often impossible to determine.

MALIGNANCY

Throughout the literature there are examples of seeding in the ventricles and subarachnoid space of the teratoid group of tumours. That the true pineal tumours can also seed is well shown in case 2, where there was intraventricular seeding, and case 4, where there was a pial deposit near the original large pinealoblastoma.

SUMMARY

Five cases of neoplasm of the pineal region occurring amongst the patients of the Royal Prince Alfred Hospital, Sydney, have been described. Of these, four are considered to be true pineal tumours bearing a certain resemblance to the embryonic epiphysis. In the first three cases this resemblance is cytological only ; in the fourth architectural. The fifth case is a teratoid growth consisting for the most part of anaplastic carcinoma with an area of typical " pinealoma " in one portion.

A classification of neoplasms of the pineal region is suggested.

PLATE III

PINEAL TUMOURS

FIG. 5.—Case 3. Mid-sagittal section of brain showing tumour.
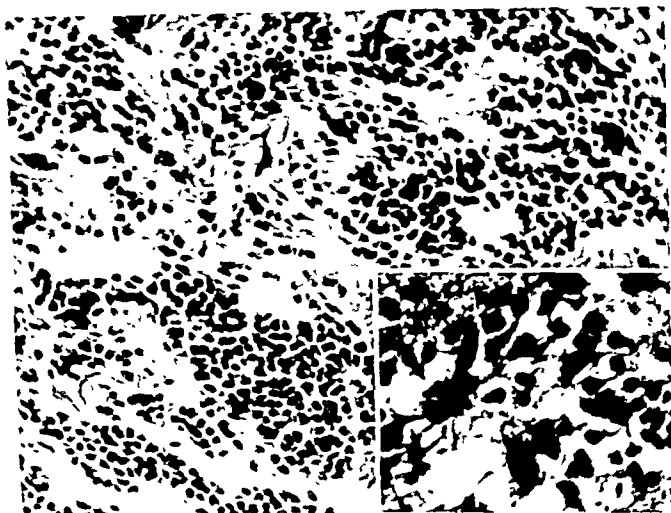
FIG. 6.—Case 3. Pinealo-blastoma. Microscopic appearance of tumour, showing its pattern. Hæmatoxylin and eosin. ×180.

Inset shows cell processes. del Río-Hortega's silver carbonate method. ×360.

FIG. 7.—Case 4. Pinealo-blastoma. Microscopic appearance of tumour : note lobular pattern. Hæmatoxylin and eosin. ×90.
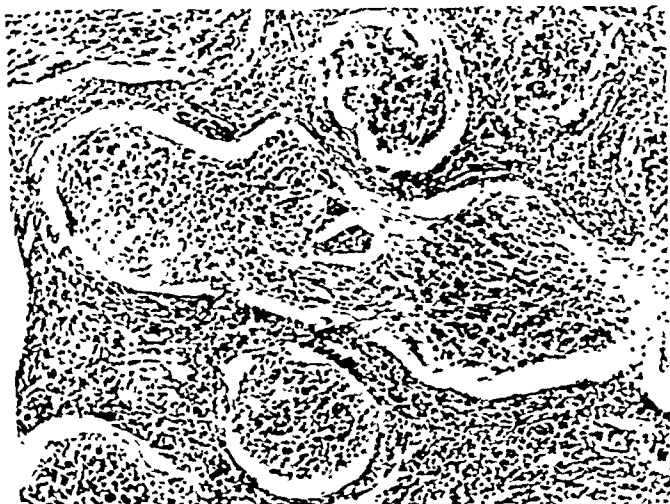
FIG. 8.—Case 4. The pattern of the reticulin network is well demonstrated. del Río-Hortega's silver carbonate method. ×90.

PLATE V

EMBRYONIC PINEAL

FIG. 9.—Epiphysis of 180 mm. embryo. Compare with fig. 3, case 2. Hæmatoxylin and eosin. ×200.
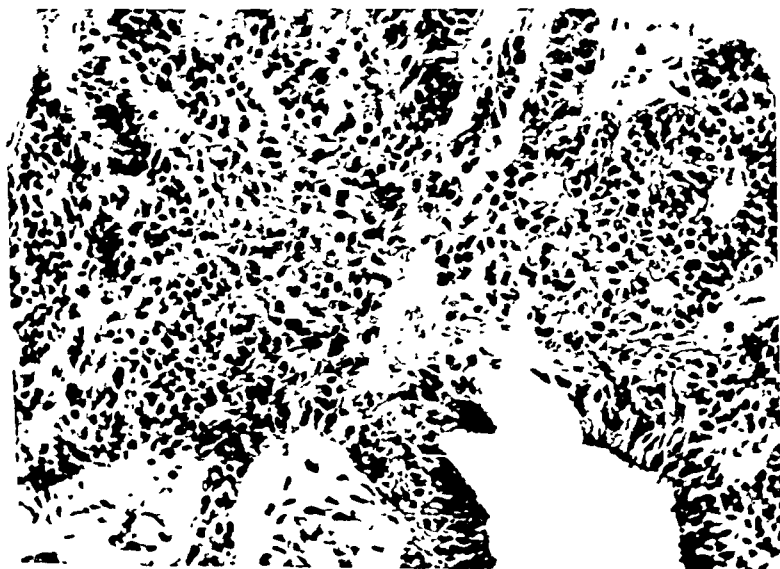
FIG. 10.—Epiphysis of 180 mm. embryo to show cell processes. Compare with fig. 5, case 2. Roger's silver method. ×200.
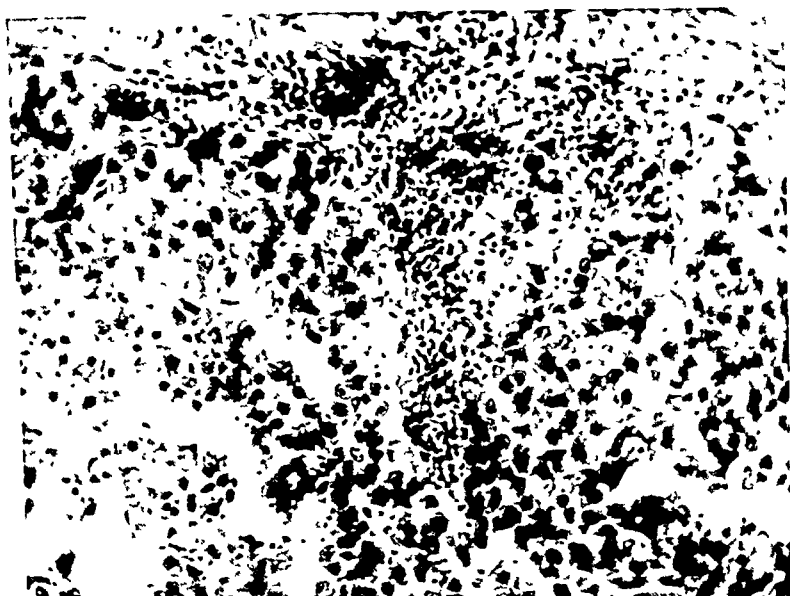
FIG 11.—Case 5 " Pinealomatous " area in tumour.   The cytoplasm of the tumour
cells is poorly defined   Hæmatoxylin and eosin.   × 200.
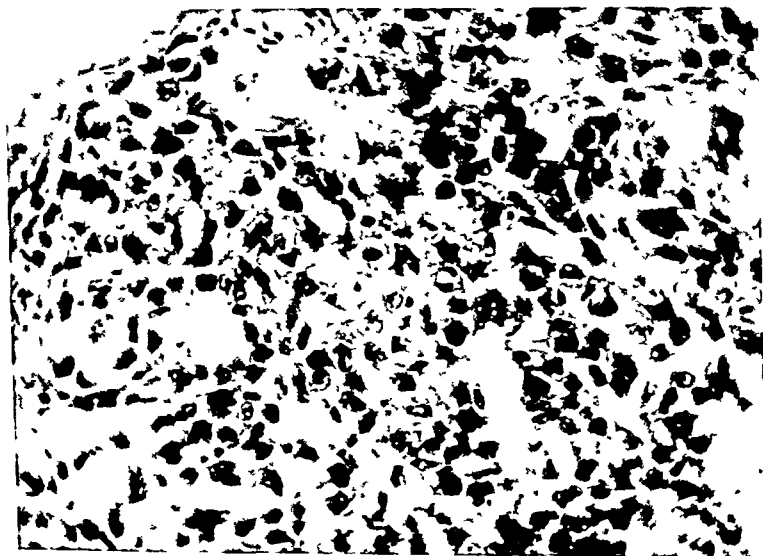
FIG. 12.—Case 5.   Another portion of the same tumour which has the appearance
of an anaplastic carcinoma   Other areas (not figured here) are papillary.   Hæma-
toxylin and eosin.   × 200.

Davies of the Fairfax Institute of Pathology, Royal Prince Alfred Hospital, kindly made available much of the pathological material. The histological preparations were made by Mr Bruce Munro of the University of Sydney. I am indebted to Mr Woodward Smith of the Medical Artistry Department, University of Sydney, for the photomicrographs.

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TERATOMAS OF THE PINEAL REGION AND THEIR RELATIONSHIP TO PINEALOMAS

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(PLATES VII-XII)

APART from true gliomas, it has been customary to recognise two distinct varieties of primary tumour affecting the pineal body : (1) new growths of bidermal or tridermal origin (teratoid or teratomatous tumours) and (2) tumours composed of large spheroidal cells and small lymphocyte-like cells, which, because they bear a resemblance to the fœtal pineal body, were designated pinealomas by Krabbe (1923). These tumours, though rare, have been thought to occur with roughly the same frequency.

In 1944, Russell presented evidence that some of the tumours described as pinealomas can be shown, on serial section, to contain a variety of structures of differing embryonic origin, and that, conversely, many typical teratomas, whether occurring in the pineal or elsewhere, contain areas of " pinealomatous " structure. She suggested, accordingly, that the commonly accepted " pinealoma " is, in fact, an atypical teratoma and that true pinealomas are very rare indeed.

Two cases are now described illustrating this relationship between teratomas of the pineal region and " pinealomas." One of the cases also presented the syndromes of diabetes insipidus and of arrested somatic and sexual development. The manner in which these features arose was clearly demonstrable in this case.

CASE REPORTS

Case I

The patient, an airman aged 23, was admitted to an Indian General Hospital in Hong Kong complaining of headache, inability to focus his eyes and double vision for the previous four weeks. There was no relevant family history nor any history of previous illnesses.

On examination at the time of admission, the patient was found to have a third nerve paralysis. The central nervous system was otherwise normal and no abnormal physical signs were found elsewhere. Lumbar puncture yielded cerebrospinal fluid which was not under increased pressure ; its constituents were normal. Blood and C.S.F. Kahn tests were negative. No malarial

parasites were found in the blood and a white-cell count and urine examination did not disclose any deviation from normal.

A week later the signs and symptoms of an upper motor neurone lesion involving the left side of the face and body became apparent. Occurring in association with the ocular signs of paralysis and gradually increasing papilloedema, this suggested that the mid-brain was the site of a tumour, but facilities for further investigation by ventriculography or intracranial exploration were not available. The patient died four weeks after admission, having progressed from drowsiness through somnolence to deep coma. A lumbar puncture terminally yielded fluid under increased pressure and containing 1000 R.B.C./c.mm.

Autopsy report

Autopsy was performed 17 hours after death. The body was that of a young adult European male, well-nourished and of good physique. There was a normal distribution of hair and the external genital organs were normally developed.

Central nervous system. The convolutions of the brain were flattened and the third ventricle was distended. On sagittal hemisection, a tumour was revealed occupying the posterior half of the third ventricle (fig. 1). It arose from the mid-line of the ventricle by a narrow pedicle containing blood-vessels originating from the tegmentum of the mesencephalon below the aqueduct. The tumour had caused considerable dilatation of the ventricle, with deformity of the corpus callosum above and softening of the thalami laterally, especially on the right. Posteriorly the tumour had stretched and thinned the posterior wall between the splenium of the corpus callosum and the quadrigeminal plate, displacing the pineal body downwards and backwards on to the superior corpora quadrigemina. The pineal itself appeared to be normal. The rostral end of the tegmentum was considerably softened and deformed by the tumour and the aqueduct was occluded. No other focus of growth was found in the ventricle or elsewhere and the remainder of the brain and its coverings appeared normal.

Examination of the thoracic and abdominal viscera did not reveal any secondary deposits of growth, nor, apart from bilateral pulmonary oedema, any other pathological abnormality.

Examination of the gross specimen revealed a firm fleshy tumour, discoloured in places by altered blood and measuring $4 \cdot 8 \times 3 \cdot 4 \times 2 \cdot 5$ cm. (fig. 2). The outer surface was irregularly lobulated and the cut surface showed this lobulation to be due to the presence of a number of cysts of various sizes. Some of the cysts contained clear fluid and others clotted blood. Many small areas of haemorrhage into the substance of the tumour were also seen.

Microscopic examination

Sections from various parts of the tumour show considerable variation in appearance. The general ground-work consists of fibrous

TERATOMAS OF THE PINEAL REGION
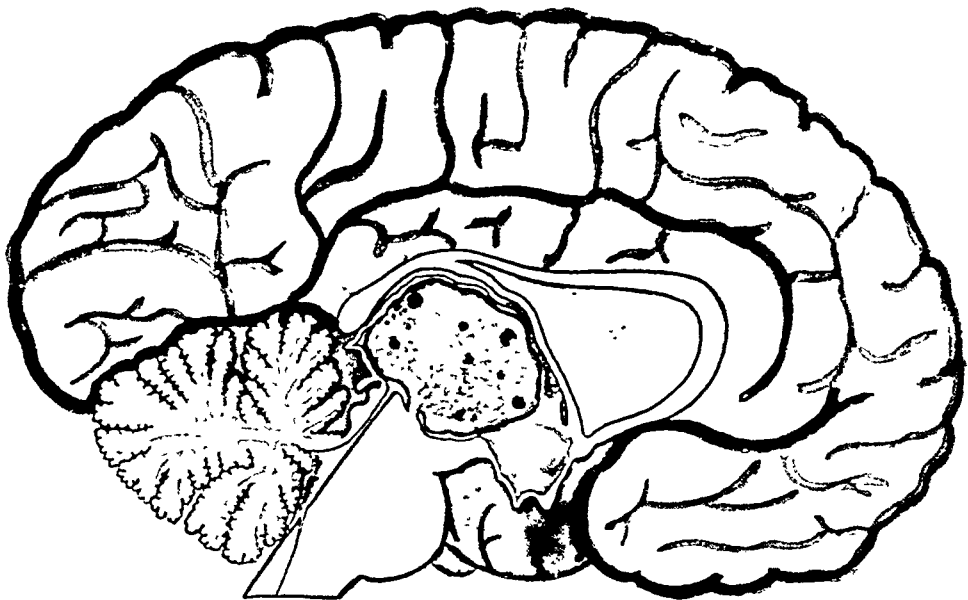
FIG. 1.—Semi-diagrammatic drawing of tumour from case I. Note normal pineal body displaced downwards and backwards on to quadrigeminal plate by tumour. Reduced.

FIG. 2.—Cut surface of tumour from case I. Note presence of many cysts, some filled with blood clot. × c. 1·4.

tissue ranging in density from coarsely collagenous to delicately myxomatous. Many blood vessels are seen, some possessing an endothelial lining and fibromuscular wall ; elsewhere there are thin-walled vessels supported only by fine loose fibrous tissue, into which hæmorrhage has often taken place. Scattered throughout the stroma is a variety of structures of differing embryonic derivation :—(1) ectodermal (stratified squamous epithelium showing keratinisation—fig. 3) ; (2) mesodermal (connective tissue, plain muscle (fig. 4) and myxomatous tissue) ; (3) entodermal (mucus-secreting columnar cells arranged in compound glandular formation and cubical and columnar epithelium arranged in simple acinar formation—fig. 5). In addition there are areas composed of large spheroidal cells interspersed with small lymphocyte-like cells, thus resembling the structure of the " pinealoma " (fig. 6).

Microscopic examination of the pineal body reveals a normal structure and there is no evidence of its invasion by the tumour.

Summary of case I

A young adult male presenting neurological signs of a tumour of the third ventricle but no psychic nor somatic sexual abnormality. Necropsy confirmed the presence of a solitary tumour in the third ventricle. Microscopy revealed a typical teratoid structure but also showed areas of " pinealomatous " structure.

Case II

The patient was first seen at the age of 18, when he was admitted to Leavesden Emergency Hospital under the care of Professor H. P. Himsworth. At this time he was found to be suffering from diabetes insipidus. He was noted then to be somewhat under-developed for his age. No cause was found for his symptoms and he was discharged on Pitressin in saline.

He was next seen three years later when he was admitted to University College Hospital, again under Professor H. P. Himsworth, with symptoms suggesting an increase in severity of his diabetes insipidus. He was then described as a pale, thin man looking at least 5 years younger than his actual age of 21 years. There was no axillary hair, scanty pubic hair and only a slight downy growth on the face. The penis and testes were small and there was general skeletal under-development, but X-ray examination of the skeleton showed the epiphyses of the long bones to be at a normal stage of fusion for his chronological age. X-ray examination of the skull showed calcification of the pineal body with no displacement. The pituitary fossa was normal. Examination of the other systems revealed a soft regular pulse of 60, blood pressure 70/48, but no other physical abnormalities. There was a slight hypochromic anæmia and a low plasma-chloride level (362 mg./100 c.c.). On Pitressin tannate in oil, intramuscularly, his fluid balance reverted to normal, his symptoms diminished and he gained weight.

The patient was lost sight of until his final admission to University College Hospital three years later. In addition to a history of anorexia, occasional vomiting and gradual loss of 2 stones in weight, he had complained of headache and failing sight over the preceding six months. He was admitted in light

coma and was extremely emaciated and severely dehydrated. Large patches of brown pigmentation were visible on his face. There was bilateral ptosis, the fundi showed bilateral primary optic atrophy, and the pupils did not react to light or accommodation. Reflex conjugate deviation was obtained in the horizontal but not the vertical plane. All four limbs were spastic, with bilateral extensor plantar responses. The pulse was soft and regular at 84/min. The systolic blood-pressure was 90 by palpation (the sounds could not be heard). Large quantities of pale urine containing no urinary chloride and of specific gravity 1002 were being passed. A mid-brain tumour was diagnosed but the patient was thought to be too ill for ventriculography. The fluid and salt deficiency were corrected and Pitressin and Eucortone were administered. The blood pressure rose to 120/70 but the signs indicated rising intracranial tension. The coma deepened and the patient died 48 hours after admission.

Autopsy report

The autopsy was performed by Dr L. E. Glynn, four hours after death. The poor development of physique and secondary sexual characteristics were again noted.

The brain was œdematous and its convolutions were flattened. The third ventricle was distended, its floor bulging down between the mammillary bodies and the pituitary stalk. The tuber cinereum and infundibulum were distended and stretched the optic tracts proximal to the chiasma. On opening the third ventricle, a tumour was seen occupying its posterior end and bulging backwards between the splenium of the corpus callosum and the quadrigeminal plate (fig. 7). The tumour arose from the anterior end of the mesencephalon and in growing backwards and downwards had caused softening and destruction of the tegmentum and obliteration of the aqueduct. Above the quadrigeminal plate, the tumour appeared to have fused with the habenular commissure, pineal body and posterior commissure and these structures could no longer be defined separately. An implantation from the tumour had occurred in the infundibulum and extended partly into the optic recess immediately above the chiasma (fig. 8). The main tumour had caused considerable distension of the third and both lateral ventricles.

The hypophysis was not increased in size but the posterior lobe appeared to be disproportionately enlarged at the expense of the pars intermedia and anterior lobe (fig. 9).

Examination of the rest of the body did not reveal any evidence of secondary deposits of the tumour but there was striking hypoplasia of all the internal organs. The heart weighed 160 g., the liver 760 g. and the kidneys together 150 g. The small and large intestine together were no more than six feet in length. Hypoplasia was especially marked in the ductless glands. The thyroid and adrenals were very small and the testes were about $1 \cdot 5 \times 0 \cdot 5$ cm. in diameter.

The proximate cause of death was moderate œdema and broncho-pneumonia of both lower lobes.

Macroscopic examination of tumour. The primary tumour was an

TERATOMAS OF THE PINEAL REGION

FIG. 3.—Case I. Area of tumour showing tongue of stratified squamous epithelium with central keratinisation. Hæmatoxylin and eosin. ×80.
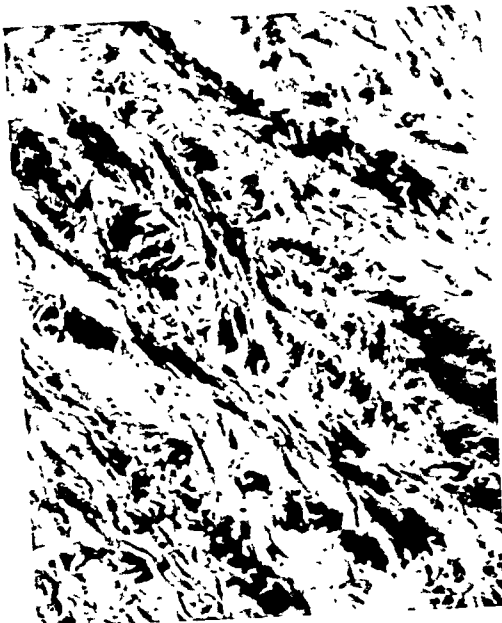
FIG. 4.—Case I. Irregular strands of plain muscle. Hæmatoxylin and eosin. ×250.

FIG. 5.—Case I. Glandular structure lined by columnar mucus-secreting cells and surrounded by a myxomatous stroma. Hæmatoxylin and eosin. ×55.
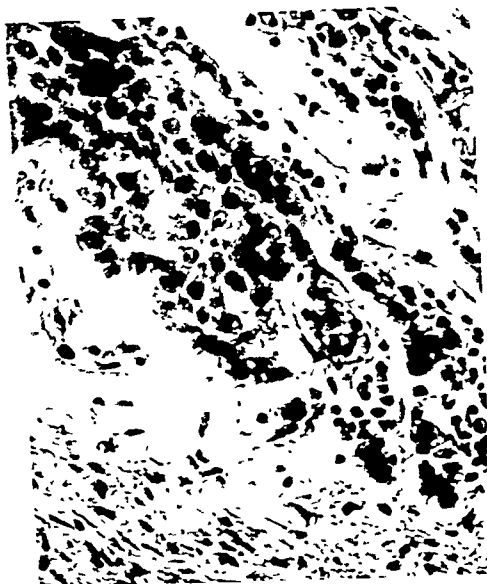
FIG. 6.—Case I. Small area of "pinealoma" cells crossing the field obliquely. Note large round or oval cells (top left) and small round cells. Hæmatoxylin and eosin. ×370.

irregularly shaped mass whose maximum dimensions were 3·4 ×
2·8 × 2 cm. It was firm in consistence and greyish-white in colour,
with scattered brownish areas of hæmorrhage and necrosis and small
glistening pearly-white foci visible on the cut surface. The seedling
from the hypothalamic region was similar in consistence and appearance
except that the cut surface presented a more uniform greyish-white
appearance and one small cystic space was visible.

Microscopic examination

Sections from different parts of the primary tumour showed the
general groundwork to consist of a loosely cellular tissue with,
here and there, areas of necrosis and scattered foci of calcification
surrounded by foreign-body giant cells. The non-necrosed tissue
was composed of two types of cell scattered diffusely throughout a
loose network of connective tissue (fig. 10). One was a large round
or oval cell with basophilic cytoplasm and a large vesiculated nucleus.
The nuclear membrane was clearly demarcated and a prominent
nucleolus was often visible. The other was a much smaller cell
indistinguishable from a small lymphocyte. The large round cells
were arranged in groups in a loose network or scattered singly in loose
clusters. The small cells were interspersed among the groups of larger
cells but were most prominent in the region of blood vessels. The
general arrangement of these cells is illustrated in fig. 11. Arranged
haphazard in the midst of this tissue, which was regarded as typical
of a pinealoma, were a variety of structures such as nodules of cartilage,
acini lined by tall columnar epithelium and small islands of squamous
epithelium (figs. 12-14).

The microscopic appearance of the seedling in the hypothalamus
(fig. 15) was similar to that of the primary tumour except for the
absence of teratomatous elements. Extension of the growth could be
traced into the infundibular stalk and anteriorly into the optic recess
above the chiasma, but posteriorly the mammillary body was not
involved (figs. 8 and 16). The neoplastic tissue could be followed
along the infundibular stalk to the posterior lobe of the hypophysis,
which also showed diffuse infiltration with the large and small round
cells. The large cells in this situation were arranged singly or in
pairs or small clusters while the small round cells were relatively
abundant and surrounded the islands of larger cells (figs. 17 and 18).
No teratomatous elements were seen. The anterior lobe of the
hypophysis was somewhat reduced in size but eosinophil, basophil
and chromophobe cells were present in approximately normal
proportions in the sections examined. Serial sectioning and a
differential count of the cell types were not undertaken.

The other ductless glands showed marked evidence of hypoplasia.
The adrenals were much smaller than normal, due to a striking
reduction in amount of the cortical tissue. The seminiferous tubules

of the testes were atrophied, those present being lined by a single layer of cuboidal spermatogonia. Spermatogenesis was suppressed, the seminiferous and efferent tubules containing merely a little colloid material. The interstitial tissue was composed largely of collagen, Leydig cells being absent or very scanty. The epididymis showed a striking hypertrophy of the fibromuscularis surrounding the efferent ducts. In general, the testes showed no evidence of gonadotropic stimulation.

Summary of case II

A young adult male showing retarded sexual and somatic development and diabetes insipidus of gradual onset and increasing severity. Terminal neurological signs of third ventricular tumour. Necropsy disclosed tumour tissue in the posterior portion of the third ventricle, with a seedling in the hypophyseal region. Microscopically the main tumour contained a mixture of teratomatous and pinealomatous elements, but the seedling in the floor of the third ventricle and the cellular invasion of the hypophyseal stalk and posterior lobe of pituitary were of pure " pinealomatous " structure.

DISCUSSION

Morbid anatomical features of teratoma and pinealoma

The occurrence and distribution of intracranial teratomas have been reviewed by Askanazy (1907), ·Barron (1916), Hosoi (1930), Harding and Naish (1935), McLean (1935), Bochner and Scarff (1938) and Ingraham and Bailey (1946). These authors have differed in their criteria of what constitutes a true teratoma, in confining or not confining their reviews only to those tumours occurring in the pineal region, or, as in the case of Ingraham and Bailey, in reporting cases from a restricted age group (children up to the age of 15).

Hosoi collected 18 cases from the literature which he described as teratomas, and a further 23 cases which he classed as teratoid tumours because derivatives of all three primitive germ layers were doubtful or absent. Of the total collection of tumours, 19 involved the pineal body and 9 the hypophysis; the others were stated to have arisen from the tela choroidea of the third ventricle, the tuber cinereum, the cerebellum and the cerebello-pontine angle. Hosoi's own case arose in the fourth ventricle. Harding and Naish's case II also arose in the fourth ventricle, their case I from the hypothalamic area.

Bochner and Scarff, confining their review to cases involving the pineal body, accepted only 13 as true teratomas and 4 as teratoid tumours, rejecting some of the cases accepted by Hosoi or describing them as undifferentiated tumours or tumours of mixed or doubtful origin. Bochner and Scarff's case was a typical tridermal teratoma arising " from the pineal region." The tumour contained an area of " pinealoma " cells which was interpreted by these authors as a remnant of the pineal gland itself, arrested in mid-fœtal development.

McLean, reviewing substantially the same field, accepted 25 cases as " pineal teratomas," but described his own case as a " parapineal teratoma," since a normal pineal body was found lying on the quadrigeminal plate. He quoted
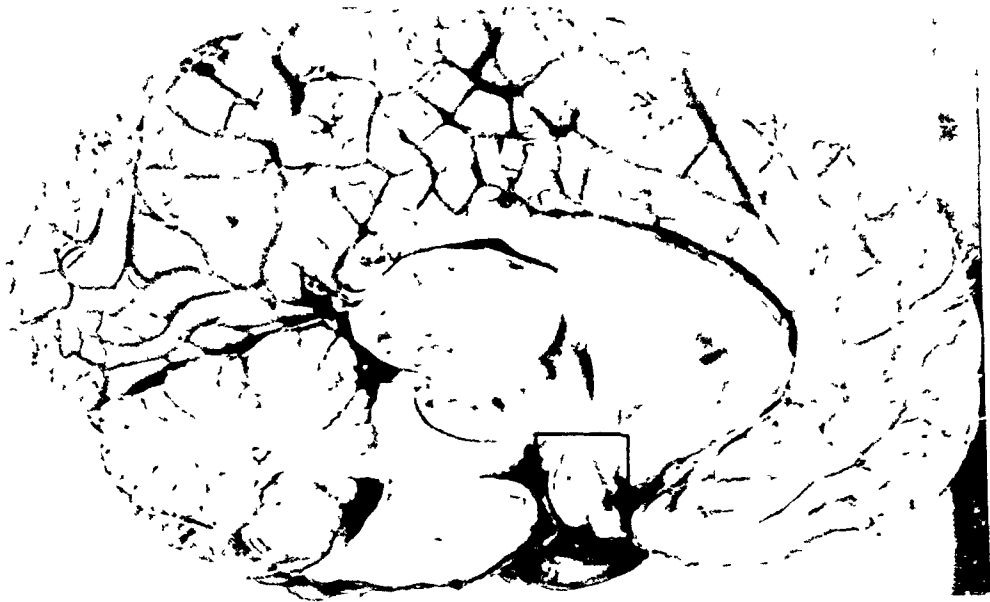
Fig 7 —Case II. The tumour occupies the posterior half of the third ventricle. Note origin from tegmentum slightly anterior to superior corpora quadrigemina; also deformity of mesencephalon below and involvement of pineal region above. Rectangle encloses seedling in hypothalamus.

FIG. 8.

FIG. 9.
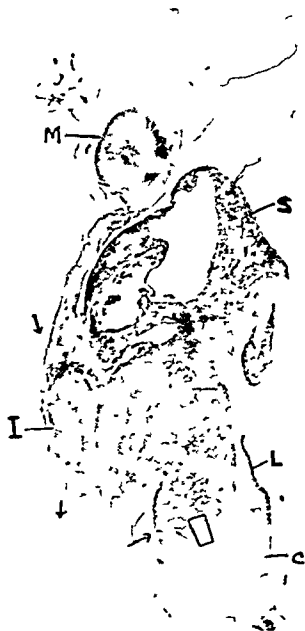
FIG. 8 —Case II. Low-power view of area enclosed by rectangle in fig. 7. Arrows indicate sites of microscopic invasion. M = mammillary body. S = portion of seedling projecting freely into ventricular cavity. L = lamina terminalis. C = optic chiasma. I = infundibular stalk. Area enclosed by rectangle shown at greater magnification in fig. 16. Hæmatoxylin and eosin. ×4.
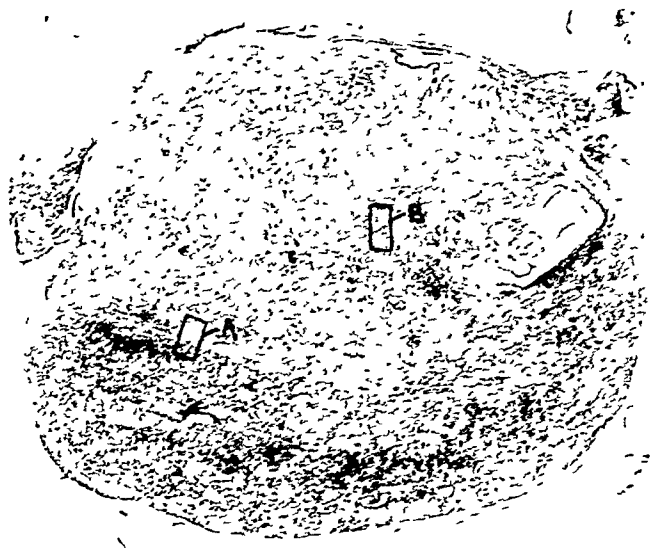
FIG. 9.—Case II. Diffuse enlargement of posterior lobe of hypophysis due to infiltration by "pinealoma" cells. The areas enclosed by rectangles A and B are shown at higher magnification in figs. 17 and 18 respectively. Hæmatoxylin and eosin.

FIG. 10.—Case II. Low-power view of section from tumour in pineal region, showing areas of "pinealoma" structure, with necrosis of tissue at left top and bottom, and rudimentary glandular structures at left centre. Hæmatoxylin and eosin. ×50.
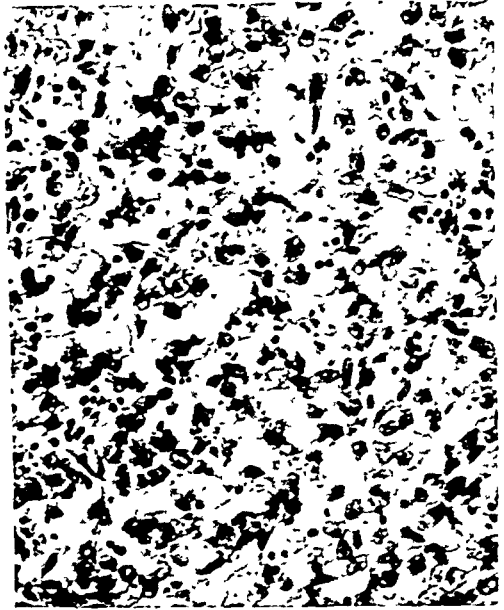
FIG. 11.—Case II. Area of "pinealoma" tissue at greater magnification, showing admixture of large and small cells. Note prominent nucleoli in large cells. Hæmatoxylin and eosin. ×210.
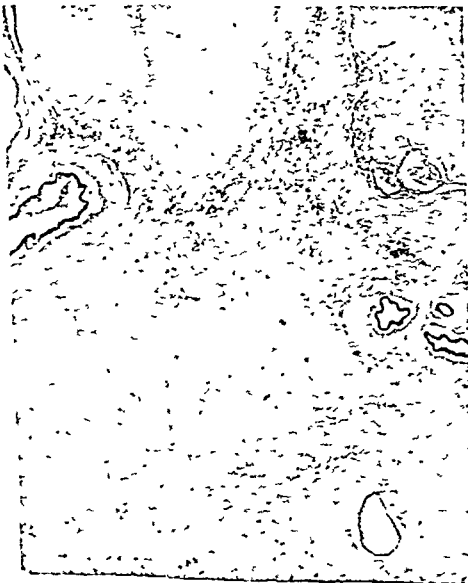
FIG. 12.—Case II. Another field showing a variety of teratomatous structures. Note acinar formations (right and left centre), cartilage (top centre), squamous epithelium (bottom right). Hæmatoxylin and eosin. × 8

FIG. 13.—Case II. From primary tumour. Acinar structure at higher magnification, showing character of lining epithelium. Hæmatoxylin and eosin. × 210.

PLATE XI

TERATOMAS OF THE PINEAL REGION

FIG. 14.—Case II. Edge of island of cartilaginous tissue (from primary tumour). Hæmatoxylin and eosin. ×250.
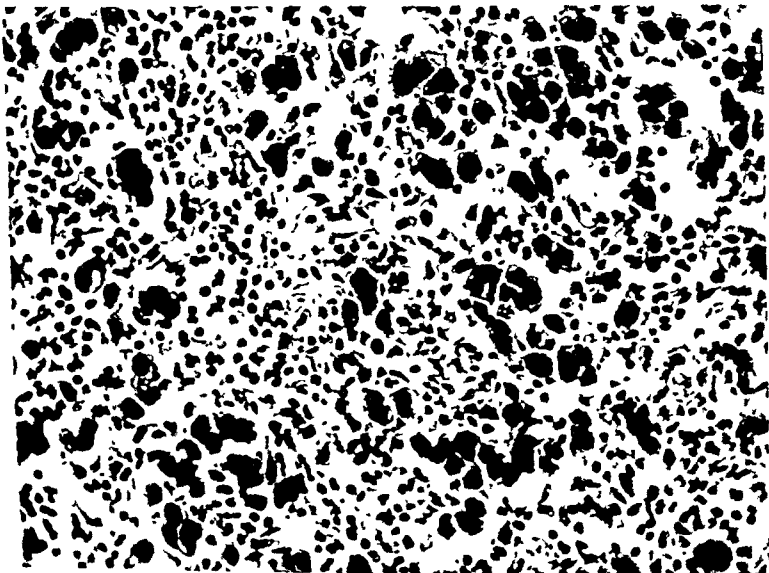
FIG. 15.—Case II. High-power view of seedling in hypothalamic area. Note increased numbers of small round cells. Hæmatoxylin and eosin. ×260.

TERATOMAS OF THE PINEAL REGION
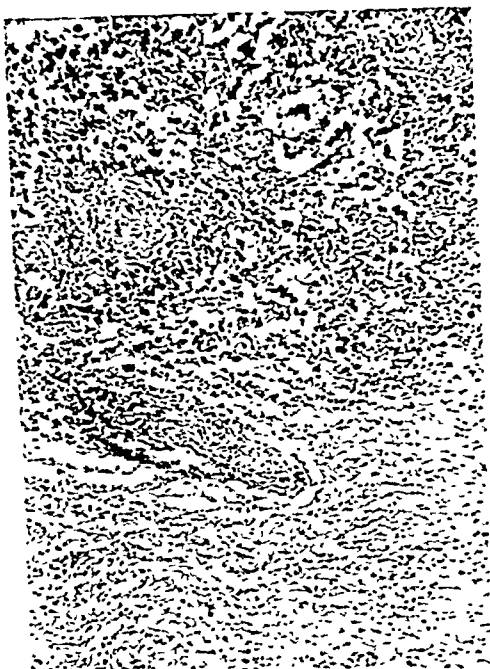
FIG. 16.—Case II. Section from area enclosed by rectangle in fig. 8, showing infiltrating pinealoma tissue above, normal chiasma below. Hæmatoxylin and eosin. ×80.
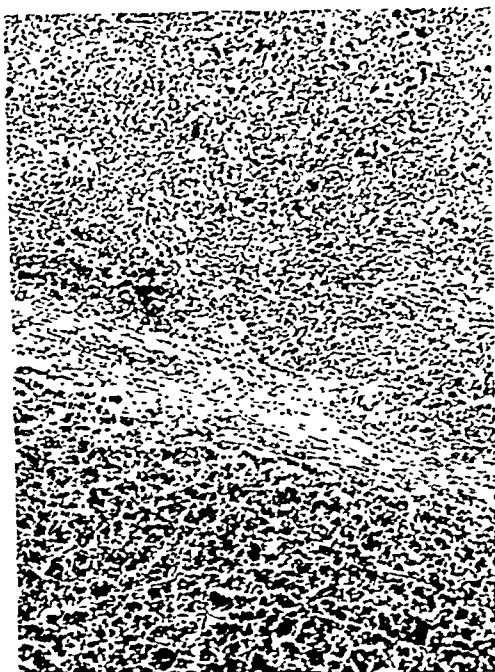
FIG. 17.—Case II. Section of hypophysis, showing normal anterior lobe below; posterior lobe infiltrated diffusely by " pinealoma " cells above. Hæmatoxylin and eosin. ×60.
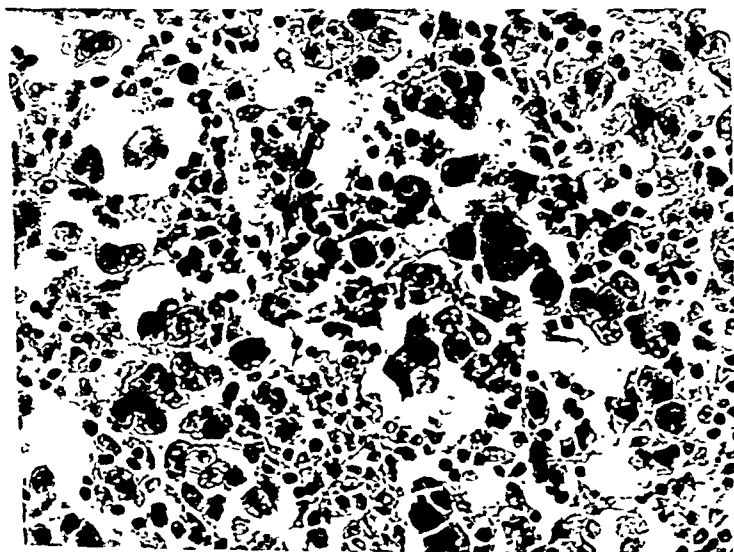
FIG. 18.—Case II. High-power view of posterior lobe, showing characteristic arrangement of " pinealoma " cells. Note prominent nucleoli of large cells. Lendrum's modification of Mallory's stain. ×370.

Ewing as mentioning that in some cases of " pineal teratoma " a normal pineal body is found and suggested that in such instances, and in particular in the case described by him, the tumour may originate from other primitive out-pouchings from the diencephalon such as the precommissural organs or the mid-habenular corpusculum parietale.

Ingraham and Bailey described 15 teratomas or teratoid tumours located within the central nervous system and occurring among 231 neoplasms of the central nervous system in children up to the age of 15 seen over a period of 20 years at the Children's Hospital, Boston. Included among these 15 cases were 8 intracranial tumours, one of which, a true tridermal teratoma, occurred within the third ventricle. Other intracranial tumours of varying degrees of differentiation occurred in the cerebellum, fourth ventricle, fronto-parietal region, choroid plexus and, bilaterally, in the frontal region. These authors also describe cases of intraspinal teratoma.

Cairns (1926) suggested that the " seminoma " or spheroidal-celled tumour of the testis might be, in fact, an atypical teratoma, and later when reporting a typical pinealoma occurring in the third ventricle, Harris and Cairns (1932) remarked upon the close histological resemblance between the pinealoma and the seminoma, adding " It may be more than coincidence that both the pineal gland and the testis, organs which are concerned with sexual function and which are known to give rise to teratomas, also give rise to spheroidal-celled tumours that bear a strong resemblance to one another ".

Pinealomas have been collected and reviewed by Horrax and Bailey (1925), Globus and Silbert (1931), Russell and Sachs (1943), Russell (1944), and Horrax (1947). All agree that the tumour originates typically in the pineal body and is commonly accompanied by seedlings in other parts of the ventricular system ; most of these authors cite the close histological resemblance of the tumour to the foetal pineal body as evidence of its origin. Russell (1944), however, presented evidence of teratomatous elements in three pinealomas examined by her, and, on the other hand, found " pinealomatous " areas in two teratoid tumours. Since then, cases have been reported by Ehni (1946) and by Glass and Culbertson (1946) in which similar " pinealomatous " areas were demon-strable in otherwise typical (tridermal) intracranial teratomas, and a similar case is cited by Willis (1948) in his section on cerebral teratomas.

Russell has also drawn attention to the occurrence of so-called " ectopic pinealomas ", quoting nine examples from the literature and adding two further cases. To these may be added case II of the series reported by Horrax. These tumours arose variously from the infundibular region, the floor of the third ventricle, the quadrigeminal plate and the region of the vermis. In each case the pineal body was normal. Russell remarks (p. 149) " . . . the occurrence and distribution of the so-called ectopic pinealoma in the brain is more easily understood if the demonstrably innocent pineal gland is exonerated ".

While typical teratomas may occur at any level in the central nervous system, arising in or close to the mid-line, the posterior end of the third ventricle appears to be an especially common site. The pineal body, because of the occasional presence within it of vestigial embryonic structures and because of its close apposition at one period of foetal development to the other primitive germ layers, has been held to be the point of origin of these tumours. The finding of a normal pineal body in some cases would make it appear that the pineal itself is not the universal site of origin of these tumours. Tumours arising in or near the mid-line from the quadrigeminal plate and extending forwards, or from the posterior wall of the third ventricle

and extending backwards, must necessarily press upon and destroy or infiltrate mid-sagittal structures such as the posterior commissure, the pineal body and the habenular commissure. The fact that these structures can no longer be identified separately, therefore, does not necessarily justify the supposition that the tumour arose primarily in the pineal.

My cases illustrate the following points in connection with these tumours :—

1. The histological relationship of teratomas and pinealomas, as emphasised by Russell, is upheld in case I, which is an example of a typical teratoma containing areas of " pinealoma " cells. Case II, on the other hand, shows a predominantly pinealomatous structure, though teratomatous elements are also demonstrable. The implantation in the hypothalamic area, in this latter case, shows a transition to a wholly pinealomatous structure and this is also evident in the infiltration of the posterior lobe of the hypophysis. Had the seedling in the hypothalamic region alone been found, there is no doubt that it would have been described as an "; ectopic pinealoma ".

2. That tumours arising in the posterior portion of the third ventricle may sometimes merely extend to and involve the pineal body rather than originate from it is also illustrated by these cases. In case I the tumour had extended sufficiently far back to cause the structures between the splenium of the corpus callosum and the quadrigeminal plate to bulge caudally, but the intact and uninvolved pineal body (as in McLean's case) was found pressed downwards on to the quadrigeminal plate. In case II, the tumour had extended even further caudally and had fused with the structures between the splenium and the quadrigeminal plate. The pineal body could no longer be identified. Nevertheless, both this tumour and that in case I clearly arose from the tegmental region.

3. The extensive involvement of the hypothalamic region, the optic recess and the posterior lobe of the hypophysis indicates the essentially infiltrative and malignant character of these lesions and supports the clinical evidence on this point. Removal of these tumours, though sometimes initially successful, has almost invariably been followed by recurrence, whether the tumour was a well-differentiated and typical teratoma, as in cases described by Ehni and by Ingraham and Bailey, or a " pinealoma ", as in cases described by Horrax.

Endocrine dysfunction occurring in association with intracranial teratoma and pinealoma

Diabetes insipidus. The occasional occurrence of diabetes insipidus in association with these tumours has not always been easily explicable, since it has not been possible to show direct involvement of the posterior lobe of the hypophysis in some cases presenting this syndrome. The earlier views have been summarised by Stringer (1933-34). Recent

experimental, physiological and clinical evidence indicating that the essential lesion is an interruption of the supra-optico-hypophyseal tract has been reviewed by Jones (1944) and discussed in relation to pineal tumours by Horrax (1947). The latter has shown that even in cases where macroscopic seedlings in the hypothalamic region have not been seen, sections have revealed microscopic infiltration of the floor of the ventricle interrupting the pathway from the supra-optic nucleus to the posterior lobe of the hypophysis.

Abnormalities of sexual development. In a review of the clinical picture presented by these tumours, Posner and Horrax (1946) refer to a small proportion of cases which show some disturbance of sexual development. In cases occurring before puberty, macrogenitosomia præcox or precocious puberty has occasionally been met with. Horrax and Bailey, in 1925, were able to collect 14 cases showing this feature. Retardation of sexual development has also been described. Rarely this may be of the dystrophia adiposo-genitalis type, or may be a generalised hypopituitarism evidenced by severe cachexia, absence of secondary sex characteristics and poor development of the genitalia. Cases 8 and 9 of Horrax and Bailey's series were of this latter type.

In spite of the equivocal evidence for the view that the pineal body serves an endocrine function in relation to sexual development, the occasional occurrence of these abnormalities of sexual development in association with tumours of this region has been held to be confirmatory of the origin of the tumours from the pineal body. The pros and cons of this argument have been fully discussed by Krabbe, Horrax and Bailey, Globus and Silbert, and Russell and Sachs.

Recent experimental, physiological and clinical evidence has been assembled by Weinberger and Grant (1941) indicating that a centre in the posterior hypothalamus in the region of the mammillary bodies is the seat of control of sexual development, its action being mediated through pathways, as yet not definitely demonstrated anatomically, to the anterior lobe of the hypophysis. Much evidence has been assembled which suggests that lesions in the region of the mammillary bodies, whether inflammatory, neoplastic or experimental, are constantly accompanied by precocious puberty in children and young animals. Moreover, 15 cases of precocious puberty in children, all of whom showed tumours involving the posterior hypothalamus in the region of the mammillary bodies, have been collected, in 12 of which the pineal body was specifically stated to be normal.

In the light of this evidence it is interesting to note that my first case, which had not involved the floor of the ventricle, did not exhibit diabetes insipidus nor any evidence of abnormal sexual development. On the other hand, when the floor of the ventricle was extensively invaded, as in my second case, diabetes insipidus was present. In this case, moreover, the mammillary bodies were uninvolved and the patient showed a kind of functional hypophysectomy. This was evidenced clinically by a general retardation of somatic development

(lack of growth hormone) ; by instability of the electrolyte balance, consistently low blood pressure and terminal pigmentation of the face reminiscent of that seen in Addison's disease (lack of adreno-corticotropic hormone) ; and by sexual hypoplasia (lack of gonado-tropic hormone). Microscopic confirmation of these changes was obtained at autopsy.

SUMMARY AND CONCLUSIONS

1. The presence of " pinealomatous " areas in a typical pineal teratoma and the transition of an atypical teratoma to a pinealomatous structure in its seedlings, support the view that many tumours in this region, reported as pinealomas, are really atypical teratomas.

2. Since similar " pinealomatous " areas occur in intracranial teratomas situated elsewhere than in the pineal and even in teratomas occurring in other parts of the body (testis and ovary), the resemblance of these areas to the fœtal pineal is not conclusive evidence of their origin from pineal tissue.

3. In some cases the pineal body is demonstrably not involved, and in these the terms " parapineal teratoma " or " ectopic pinealoma," which suggest an origin from the pineal body, are not justified. In other cases close examination of the tumour often suggests that an inert pineal body has become involved, by virtue of its anatomical position, in a tumour originating nearby from the posterior end of the third ventricle or from the quadrigeminal plate. Here again a primary pineal origin is doubtful.

4. These tumours are often invasive and microscopic involvement of the floor of the third ventricle is often of greater extent than naked-eye examination would suggest. Interruption of the supra-optico-hypophyseal tract in this manner accounts for the occasional production of diabetes insipidus. The occasional occurrence of abnormalities of sexual development in association with these tumours is probably also due, not to the involvement of the pineal body, whose endocrine function is doubtful, but to spread to the hypothalamic area or the hypophysis.

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AN EXPERIMENTAL STUDY OF THE RELATION-SHIP BETWEEN PLASMA PROTEINS AND LIVER DISTURBANCE *

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SOME investigators maintain that the liver is the main, if not the only source of the plasma proteins, or that the liver is the chief storehouse for these proteins, doling them out into the blood as the need arises. But there is a group of workers who are not so certain about this relationship, and who hold that a specific reservoir for proteins cannot as yet be named. For them, there exists a labile association between many of the body tissues and the blood proteins, and there may be no limit to the types of cells which can be drawn upon for proteins when the emergency arises. In this paper I re-consider the problem of plasma-protein origin, with especial attention to the theory of hepatic origin, based on new experimental work carried out on animals during the last two years.

METHODS

Rabbits and rats were subjected to a variety of procedures which are well known to induce hepatic damage or insufficiency, namely :—

(a) Single and repeated subcutaneous administration of carbon tetrachloride, which leads to acute liver necrosis and cirrhosis.

(b) Repeated cutaneous application of coal tar, which causes necrosis and chronic liver damage ending in cirrhosis.

(c) Repeated oral administration of butter-yellow, which leads to cirrhosis and tumour-formation in the liver.

(d) Ligation of the common bile duct with prolonged interruption of bile excretion.

(e) Establishment of an Eck fistula to reduce the inflow of portal blood.

(f) Hepatectomy.

The plasma-protein concentration of the experimental animals was followed at intervals, liver function tests based on variations in plasma-protein fractions (thymol turbidity, colloidal gold and, in rabbits, the Takata-Ara reaction) being performed simultaneously. Finally the biochemical behaviour was correlated with the structural alterations in the liver.

Further experiments included a study of the rate of regeneration of plasma proteins after bleeding in Eck fistula rats and after the administration of carbon ink and carbon tetrachloride, together with some observations on the influence of general metabolic disturbances and impaired nutrition caused by dinitro-*o*-cresol on plasma-protein concentrations and on liver function tests.

Animals

Altogether 177 rats and 44 rabbits were used in these experiments.* There was a fairly high incidence of unavoidable casualties in some experiments, especially after severe operative procedures. Both rats and rabbits were of uncertain stock, about two-thirds being obtained from outside dealers and the rest bred from our own stock. Those from external sources were acclimatised in our animal house for at least three weeks before use. All appeared healthy at the beginning of the experiments. No discrimination was made between sexes. Rats weighed from 110 to 325 g. with the majority about 200 g., rabbits from 1·7 to 3·6 kg.

Diet

Rats. Three diets were used for different experiments. (1) The first was the M.R.C. diet VII, supplied in cubes of the following composition :

Rolled oats or ground oats	.	.	.	30 per cent.
Wholemeal flour	.	.	.	.	35 ,,
Dried yeast .	.	.	.	.	6 ,,
Dried skimmed milk	.	.	.	15 ,,
Meat and bone meal	.	.	.	10 ,,
Cod-liver oil .	.	.	.	.	2 ,,
Sodium chloride .	.	.	.	1 ,,
Calcium carbonate	.	.	.	1 ,,

This diet was given in liberal quantities, with an unlimited supply of drinking water and frequent supplements of green vegetables in all experiments with the exception of the following two investigations.

(2) In the dinitro-*o*-cresol experiments the Rowett Institute Diet (Thomson, 1936) was used. The cubes were pulverised in a mortar and mixed with dinitro-*o*-cresol solution to the required concentration. During the first month the diet was given without limitation, after which each rat received only 15 g. per day. Drinking water was supplied *ad lib.* and green vegetables were given frequently.

(3) In the butter-yellow experiments, the animals were fed on a mixture of

Unpolished rice	.	.	.	.	1 kg.
Pea-nut oil .	.	.	.	.	30 c.c.

with a liberal supply of drinking water. They also received 1 g. of fresh carrots every morning except on Sundays. The protein content of this mixture is less than 1 per cent.

Rabbits. The rabbits received unlimited amounts of stock diet in pellets of the following composition.

Grass meal .	.	.	.	.	30 per cent.
Ground-nut cake .	.	.	.	15 ,,
Barley meal or sugar-beet pulp	.	.	20 ,,
Calcium carbonate	.	.	.	1 ,,

This was supplemented with hay and drinking water.

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* Full tables are included in the original thesis, which is deposited in the University of London Library.

Procedures for inducing liver damage

Administration of carbon tetrachloride. (i) Three rabbits were given a single subcutaneous dose of 1 c.c. per kg. body weight of carbon tetrachloride (Analar).

(ii) Six rabbits received daily subcutaneous injections of 1 c.c. per kg. body weight of carbon tetrachloride. Three animals were killed on the third day. In the remaining 3 the injections were continued until they died or became very weak, when they were killed.

(iii) Thirteen rabbits were given bi-weekly subcutaneous doses of carbon tetrachloride. The initial dosage was 0·5 c.c. per kg. body weight. As the animals acquired more tolerance to the poison, the dosage was increased from time to time. Towards the end of the experiment the survivors were tolerating 4 c.c. per kg. body weight. With rapid deterioration of the animal, the dose was decreased or even discontinued, but resumed when the animal improved in condition. The aim was to maintain the animals in a toxic state with gradual loss of weight until cirrhosis of the liver was established. Strict asepsis was maintained in giving the injections but abscesses developed in the later periods in the injected areas of some animals. The experiment lasted 38 weeks. Body weights were recorded at regular intervals.

Application of coal tar. Sixteen rabbits were employed, 11 of which were tarred and 5 served as controls. Coal tar obtained from a local shop was applied to the external surface of one ear in 5 rabbits and to the shaved skin of alternate sides of the back in 6 rabbits. Tarring was done twice a week up to 16 weeks and approximately 2 c.c. were applied each time. Accumulations of dried and hardened tar often required to be removed before fresh applications were made.

Administration of butter-yellow (*β-dimethylaminoazobenzene*). Butter-yellow was dissolved to a concentration of 3 per cent. in peanut oil with the help of gentle heating, 30 c.c. of this solution being intimately mixed with 1 kg. of unpolished rice and supplied in generous amounts to the 32 experimental animals. The control rats received the same diet without the butter-yellow. All the animals were given supplements of fresh carrot (1 g. per day) and water *ad lib.* All lost weight and in the later stages showed loss of hair, dermatitis and scaly skin.

Ligation of the common bile duct. The method of Cameron and Oakley (1932) was employed. Forty rats survived the experimental procedure. Sham operations were performed on 27 controls. Groups of rats were killed with ether at intervals ranging from 5 to 90 days after collecting blood samples by cardiac puncture. The animals were weighed at regular intervals. Six rabbits underwent the operation successfully but the mortality from ruptured gall bladder and bile peritonitis was very high.

Eck fistula. This was successfully produced in 13 rats by the ingenious method of Whitaker (1946). The controls underwent a sham operation. The daily food intake of the Eck fistula rats was noted and the same amount fed to the control animals. No special after-care was necessary if the operation proved successful. The technique of Glynn and Himsworth (1946-48) of slowly injecting 1 c.c. of freshly filtered Mandarin black into the spleen, after opening the abdominal cavity under ether anæsthesia, was used for studying the hepatic circulation in 5 experimental and 3 control rats.

Hepatectomy was performed on 11 rabbits by the method of McMaster and Drury (1929). The animals fasted overnight but were given an adequate supply of drinking water. Before operation and after collecting a sample of blood they were given 10 c.c. of 10 per cent. glucose solution intravenously. After operation the animals were moved to a warm dark room and kept as quiet as possible, as any disturbance may precipitate convulsions followed by death. An intravenous drip of 10 per cent. glucose solution into the marginal vein of an ear was started at once. The needle was held in place by two clips and the ear was stitched to the skin of the back. Each animal received about 1 c.c.

of glucose solution per minute. The drip required occasional adjustment, as it gradually decreased in rate. Two rabbits were hepatectomised without subsequent glucose administration. Six controls underwent a sham operation and about 10 c.c. of blood were removed during this procedure. Glucose solution was then administered in the way described above. Blood samples were taken at intervals from all animals. Great difficulty was experienced in bleeding the hepatectomised animals in their terminal periods.

Bleeding, with and without carbon ink and carbon tetrachloride injections and Eck fistula. Twenty-five adult rats were bled by a modification of the technique of Cutting and Cutter (1935). Cardiac puncture was used for bleeding and the blood samples were heparinised. The procedure was performed on :— (1) six rats after intraperitoneal injection of 1 c.c. of carbon ink ; (2) five rats after subcutaneous injection of 0·2 c.c. carbon tetrachloride per 100 g. body weight ; (3) five rats after the establishment of an Eck fistula by the method already mentioned ; (4) nine control rats.

Administration of dinitro-o-cresol. Eleven large adult rats weighing 280-325 g. were used. Dinitro-o-cresol (0·25 g.) was dissolved with 0·12 g. sodium bicarbonate in 100 c.c. of distilled water. Rowett Institute rat cubes were pulverised and the dinitro-o-cresol solution was intimately mixed with 1 kg. of the powder, giving a final concentration of 0·025 per cent. (w/w) dinitro-o-cresol in the diet. Unlimited amounts of this mixture together with drinking water *ad lib.* and frequent supplements of green vegetables were supplied to rats for a month. They ate voraciously but lost weight. As no variation was noted in their plasma-protein concentration at the end of this time they were subsequently maintained on 15 g. of food per rat per day, and the concentration of the dinitro-o-cresol was gradually raised from 0·025 to 0·05 per cent. This produced a tremendous reduction in body weight. Controls were used after the first month, and these received about the same quantity of food as the experimental animals.

Biochemical methods

In rabbits, blood samples were collected directly from the marginal vein of the ear into dry centrifuge tubes : in rats, cardiac puncture was performed. Serum was collected after clotting and centrifugation at 3000 r.p.m. for 20 minutes. In the experiments on regeneration of plasma proteins, heparinised plasma was used. Estimations of total protein, albumin, globulin and non-protein nitrogen were made by the micro-Kjeldahl method of Rimington and Bickford (1947).

The thymol turbidity and serum colloidal gold tests were performed on rats, using the methods of Maclagan (1944a and b). In rabbits, Ucko's modification (1942-43) of the Takata-Ara test was done in addition.

The direct Hijmans van den Bergh reaction was followed in animals whose common bile ducts had been ligatured.

Positive human sera from the clinical pathological laboratory of University College Hospital served as a check on the sensitivity of the reagents for the three liver-function tests.

Histological methods

At the end of the experiment, or when they were moribund, the animals were quickly killed with ether and a careful autopsy was performed. Particular attention was paid to the macroscopic appearance of the liver, spleen and portal system, and a collateral circulation and ascites were looked for. The liver was weighed and often a sketch of the lesions was drawn. Samples of tissue up to 3 mm. thick were taken from all lobes of the liver, spleen, duodenum, ileum, kidneys, heart, adrenal glands, mesenteric lymph glands, bone marrow of femur, extensor muscles of thigh, often brain and any tissue which appeared abnormal on naked-eye examination. At least seven samples were removed

from the liver of each rat and about a dozen from rabbits. These were fixed in 10 per cent. formol-alcohol and 10 per cent. formol-saline. After twenty-four hours they were trimmed and fixed in fresh fixatives for a further twenty-four hours. Material from the 10 per cent. formol-alcohol was dehydrated in graded alcohols, cleared in chloroform and embedded in paraffin. Frozen sections were cut from tissue fixed in 10 per cent. formol-saline and stained for fat with Scharlach R. or Sudan III with Ehrlich's acid hæmatoxylin as the counterstain. The paraffin sections were stained with Ehrlich's acid hæmatoxylin and eosin and Weigert's iron hæmatoxylin and van Gieson's stain.

Sections of the liver were examined by an independent person without any knowledge of the experimental details of the animals and a very conservative assessment of the extent of damage was made.

RESULTS

Subcutaneous administration of carbon tetrachloride

Six rabbits which received 2-4 successive daily doses subcutaneously showed hepatic damage involving $\frac{1}{2}$-$\frac{3}{4}$ of the liver parenchyma. Necrosis of hepatic cells around the central veins was prominent; in some areas they had disappeared, leaving behind only the mesenchymal stroma. The more peripheral cells were hydropic or fatty. Regenerative activities were not evident. Moderate infiltration by neutrophils, histiocytes and lymphocytes was seen in the portal tracts and necrotic areas in some cases.

Thirteen rabbits received bi-weekly subcutaneous injections of carbon tetrachloride over 3-38 weeks. The resulting liver destruction was conservatively estimated as up to 50 per cent. of the liver substance. The earlier stages included centrolobular necrosis and degeneration, often with mitosis and other signs of regeneration in the hepatic cells. With continued action of the poison, fibrosis became apparent. Eventually the surviving animals developed a pale, tough and often nodular liver, with fibrous bands separating the parenchyma into islands of liver cells of various sizes. Some of these were regenerative, with loss of the normal architectural pattern. Fatty and hydropic degeneration and necrosis were also seen in some nodules. Frequently bile-duct hyperplasia and cellular infiltration of the fibrous tissue were pronounced. The whole picture now was strongly reminiscent of human portal cirrhosis.

Changes in other organs were frequent and striking. The kidneys often showed marked tubular degeneration and even necrosis. A peculiar multilocular cystic change in the wall of a segment of the large intestine was observed in 3 rabbits. The cysts were empty and smears from them were negative. Hæmorrhages were seen in the retroperitoneal tissue of 3 rabbits, hæmorrhage and necrosis in the adrenal glands of one rabbit and petechial hæmorrhages in the pericardium of one rabbit. Other lesions included stenosis of the ileum with thickening of the wall and submucous necrosis (one case), myocardial degeneration and fibrosis (one case), and multiple acute ulcers

in the gastric mucosa (one case). Altogether, 16 out of 19 animals presented pathological changes apart from the liver.

Biochemical changes. Despite much destruction of liver cells following injections of carbon tetrachloride (conservatively estimated as up to 75 per cent. of the organ), the plasma-protein concentration remained within the range of normal variation (fig. 1). Liver function tests also showed no deviation from the normal.
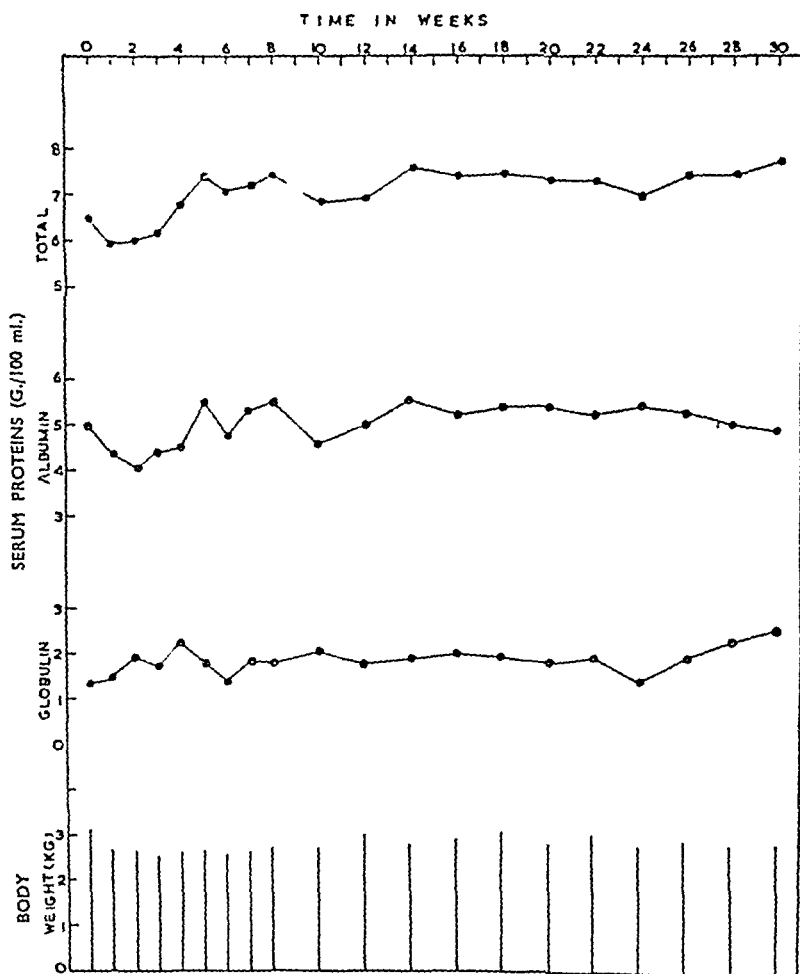
FIG. 1.—Effect of repeated subcutaneous administration of carbon tetrachloride on the serum-protein concentration and body weight in a representative rabbit.

Skin application of coal tar for 1-16 weeks

Most of the animals lost weight noticeably and some were very emaciated towards the end. Structural changes in the liver corresponded closely with those described by Davidson (1925). Acute effects included necrosis of liver cells, sometimes involving extensive areas, with adjacent hydropic and fatty degeneration and hæmorrhages.

Necrotic tissue was often infiltrated by polymorphs, lymphocytes and histiocytes. Animals which survived the treatment developed chronic changes. The liver then appeared pale and firm and sometimes showed a granular surface. Microscopically there was hyperplasia of fibrous tissue in all stages to well-formed broad bands dividing the parenchyma into groups of liver cells. Nodules of regenerated tissue, bile-duct proliferation, necrosis and degeneration were also present. The liver damage assessment varied from very slight to 80 per cent.

• — TAR
○ – CONTROL
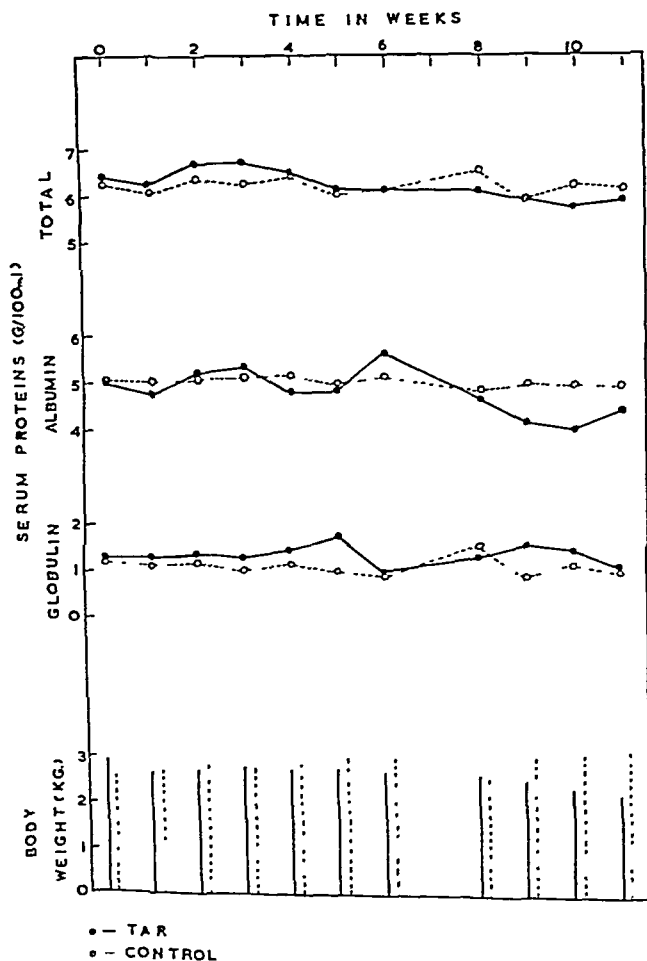
FIG. 2.—Effect of repeated skin application of coal tar on the serum-protein concentration and body weight. Mean of six experimental and four control rabbits.

Two rabbits showed fatty degeneration of the renal tubules and one a large retro-peritoneal hæmorrhage ; the other animals showed no abnormal extra-hepatic disturbance.

No definite relation could be established between the extent of liver damage and the serum-protein concentration in tarred rabbits (fig. 2). Liver function tests were negative, although the hepatic damage affected up to 80 per cent. of the liver parenchyma.

Administration of butter-yellow

The structural changes in the liver in my animals agreed closely with those noted by Orr (1940) and Opie (1944) and included necrosis, degeneration and regeneration of liver cells, proliferation of bile ducts and cellular infiltration. In later periods the liver showed fibrosis, regeneration nodules, patches of necrosis and degeneration of liver cells and hyperplasia of bile ducts. Sometimes the bile ducts were
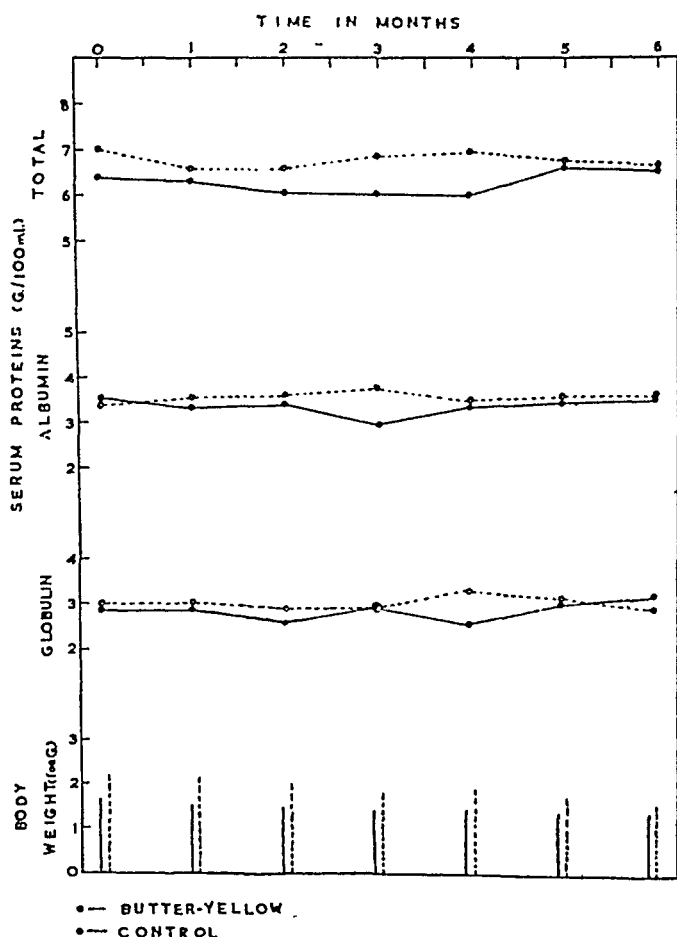
Fig. 3.—Effect of repeated oral administration of butter-yellow on the serum-protein concentration and body weight. Mean of six experimental and six control rats.

enormously dilated. Tumours were frequent and included liver-cell carcinoma, bile-duct carcinoma and bile-duct cystadenoma, often in combination. The extent of liver damage varied a great deal, the most severe type affecting eleven-twelfths of the liver substance.

Fig. 3 gives the mean plasma-protein concentration and body weight of six rats compared with their controls during a period of

six months. There were no variations which could be considered significant. An occasional inversion of the albumin-globulin ratio was noted. Since this was sometimes present before the administration of butter-yellow and in the controls it could not be attributed to the liver damage caused by the butter-yellow. It should be noted, as mentioned before, that the diet was low in protein and of poor nutritive value, yet the plasma-protein concentration level was maintained in both experimental and control groups.

Liver function tests also gave disappointing results. Three experimental animals showed positive colloidal gold reactions even before the administration of butter-yellow, so that the value of the test was questionable. The thymol turbidity test was consistently negative.

Ligation of common bile duct

In rats, the common bile duct became dilated, often attaining an enormous size. The essential changes in the liver were focal necrosis irregularly distributed and in various stages of development, hyperplasia of bile ducts, beginning in the portal tracts and later reaching an extraordinary degree in many cases, with disorganisation of the lobular structure, cellular infiltration of varying extent, and degeneration and regeneration of the liver cells. Formation of fibrous tissue was usually inconspicuous but occasionally marked. The bile ducts were often dilated when duct proliferation was extensive, and the liver cells were then atrophic and large numbers disappeared. The liver damage was assessed as ranging from slight to 88 per cent. of the parenchyma. Some rats survived for 90 days but most died from rupture of the common bile duct 5-60 days after operation. The serum-protein concentration showed no significant differences before and after these intervals.

Of the rabbits, one showed a significant fall in serum-protein concentration on the 24th day, but the animal was moribund when the blood sample was collected and had been off its food for several days. The liver damage amounted to 50 per cent., whereas rats with up to 88 per cent. and a rabbit with 84 per cent. damage showed no such change.

In rabbits, the liver function tests were all negative. Rats gave a negative reaction to the thymol turbidity test. Three reacted positively to the serum colloidal gold test before and after duct ligation. One rat gave a positive colloidal gold reaction before ligation, which disappeared after the operation. Some controls also gave a positive reaction to the colloidal gold test.

Eck fistula

Histological examination of the liver revealed little alteration after the formation of an Eck fistula. Some diminution of blood-flow through the liver could be established by intravital injection of ink.

No significant alteration in serum-protein concentration was detected (fig. 4). A slight fall in albumin concentration could be ascribed more probably to extrahepatic factors than to liver disturbance. Liver function tests were found to be unreliable.
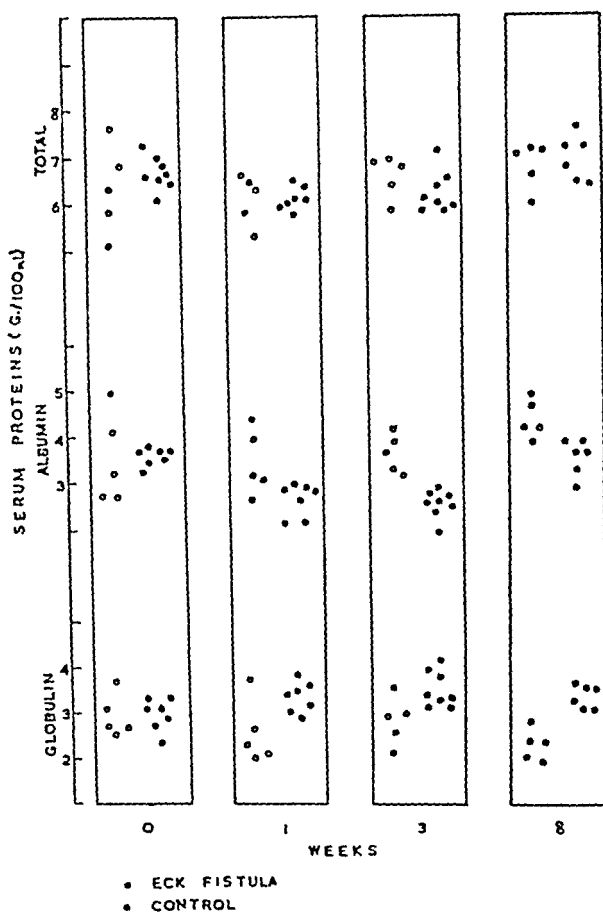
Fig. 4.—Effect of Eck fistula on the serum-protein concentration in rats.

Hepatectomy

Animals on glucose made a rapid recovery from the anæsthetic. They remained noticeably quiet, refusing food and even water and appeared very apprehensive. Muscular weakness gradually became evident and the animals were prostrated towards the end. Respiration was rapid and shallow and in later stages often laboured and noisy. Two had convulsions before death but the others died quietly with respiratory failure. The survival period ranged from 11 to $25\frac{1}{2}$ hours. The two rabbits not given glucose remained quiet but appeared anxious. Muscular weakness gradually supervened. Convulsions occurred suddenly and without warning. They died $3\frac{1}{2}$ and 4 hours respectively after the operation.

At autopsy the remaining caudate lobe was occasionally congested but usually pale, and very soft and friable. The presence of blood clot was frequent at the operation site but no severe signs of portal stasis were found.

Microscopic examination of the remaining caudate lobe showed severe necrosis and degeneration of the liver cells; frequently only those bordering the portal tracts appeared to be still alive. The extent of hepatic insufficiency was estimated to be 95-100 per cent.
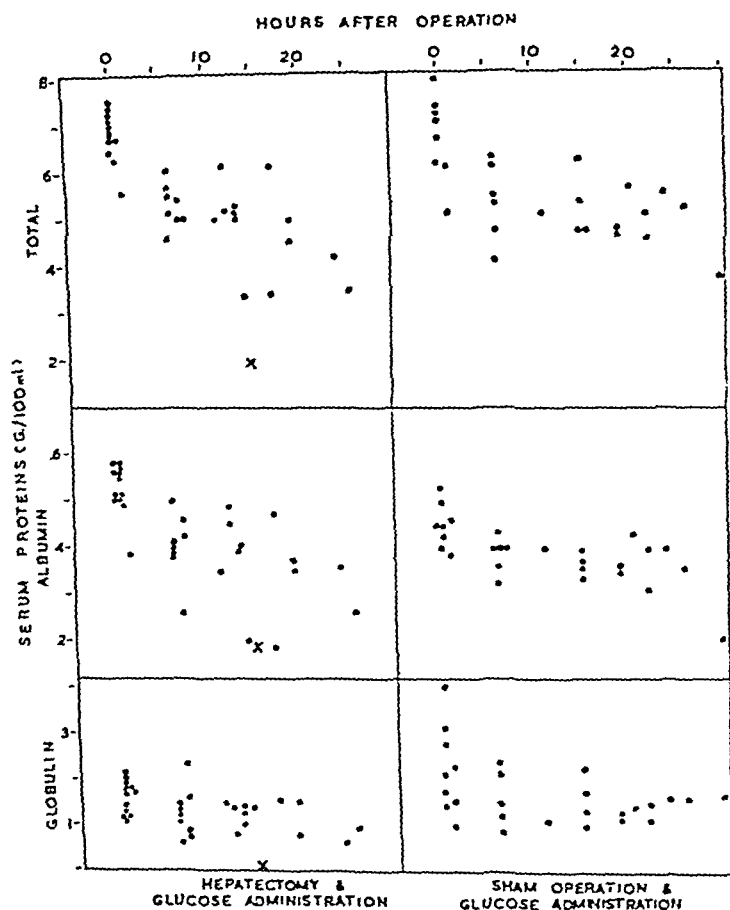
FIG. 5.—Effect of sub-total hepatectomy and sham operation followed by continuous intravenous glucose administration on the serum-protein concentration in rabbits. x = after post-mortem glucose infusion.

In the absence of glucose treatment the serum proteins and hæmoglobin percentage had not altered when the animals died of hypoglycæmic convulsions in 3½-4 hours. If hepatectomy was followed by continuous administration of glucose solution both serum-protein concentration and hæmoglobin percentage dropped. This fall was most marked with the serum albumin. However, similar changes were observed in the controls (fig. 5). Assuming that hæmoglobin

percentage is an index of the extent of hæmodilution by glucose administration, plotting the corresponding serum-protein concentration against it showed a close relationship between hæmoglobin percentage and the serum-albumin concentration after both hepatectomy and a sham operation. The serum-globulin concentration showed a greater degree of scatter. Berryman, Bollman and Mann (1943) noted an increase in pseudoglobulin in hepatectomised dogs after glucose administration and this may have been the case in my experiments. Hence one feels justified in interpreting this change as associated with the glucose administration, and perhaps with circulatory readjustment and other complications caused thereby as well as the effects of anæsthesia and operative interference, rather than due to removal of the liver.

Regeneration of plasma proteins after bleeding, combined with (i) intraperitoneal injection of carbon ink, (ii) subcutaneous injection of carbon tetrachloride, (iii) Eck fistula

(i) Bled rats given an intraperitoneal injection of 1 c.c. of carbon ink showed a striking fall in both plasma-protein concentration and total circulating proteins (fig. 6). The albumin fraction was mainly involved. These results agree with those obtained by Cutting and Cutter. No definite indications of the mechanism of the delay in the regeneration of plasma proteins after bleeding in rats injected intraperitoneally with carbon ink could be obtained from the pathological examination. Usually some ink still remained in the peritoneal cavity, especially in relation to the omentum. The diaphragmatic lymphatics were often distended with ink particles. The most constant histological feature was the pronounced phagocytic activity of the cells of the reticulo-endothelial system for ink particles. This was especially the case in the lymph glands, although even here the effect was somewhat inconstant. Phagocytosis of the ink particles by the Kupffer cells and by the macrophages of the spleen was striking in two cases. Diaphragmatic and mesenteric lymph vessels contained ink particles in one case. Obviously more work needs to be done on this interesting problem before a satisfactory answer can be found.

(ii) Sections of the liver from the five rats given subcutaneous injection of 0·2 c.c. per 100 g. body weight of carbon tetrachloride showed typical centrolobular necrosis fringed with hydropic and fatty degeneration, about ¾ths. of the tissue being abnormal. In each instance the plasma-protein concentration had returned to the initial level 12 hours after bleeding and the total circulating protein in general agreed with that in the controls (fig. 6).

(iii) In Eck fistula rats the plasma-protein concentration regained its initial value in 12 hours and the total circulating protein also varied little from that of the controls (fig. 6).

(iv) Controls. Twelve hours after bleeding, all controls had practically regained or even exceeded their initial plasma-protein concentration and total circulating protein (fig. 6).
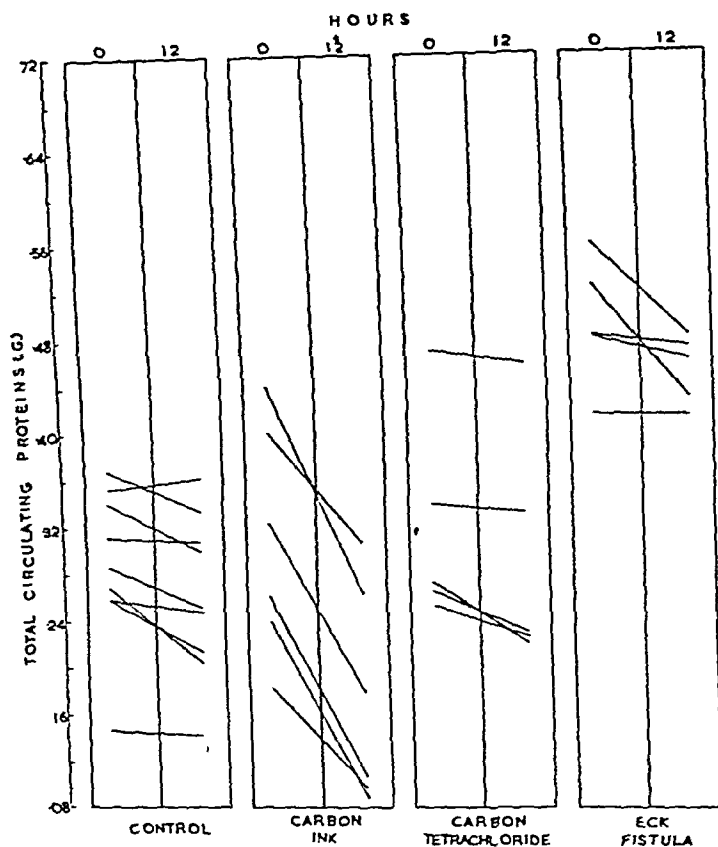
FIG. 6.—Effect of intraperitoneal injection of carbon ink, subcutaneous injection of carbon tetrachloride and Eck fistula on the total circulating plasma protein 12 hours after severe bleeding in rats.

Administration of dinitro-o-cresol

The most striking feature in this experiment, which extended over 76 days, was the tremendous loss of weight. Autopsy revealed a wasted body and atrophic viscera. No histological abnormalities were found in the organs.

The total serum-protein concentration of one rat was reduced to as little as 3·75 g. per 100 ml. when examined on the 61st day. Two others showed a less severe fall. These results cannot be accepted without reserve, for the animals were moribund when the blood samples were collected, and the two controls, which were found moribund, also showed a reduction in serum-protein level. Hence the fall was most likely to be due to the deficient food intake and the moribund state of the animals and not to the metabolic disturbance

caused by the dinitro-*o*-cresol.   Other animals in these groups showed
no significant changes in protein concentration (fig. 7).
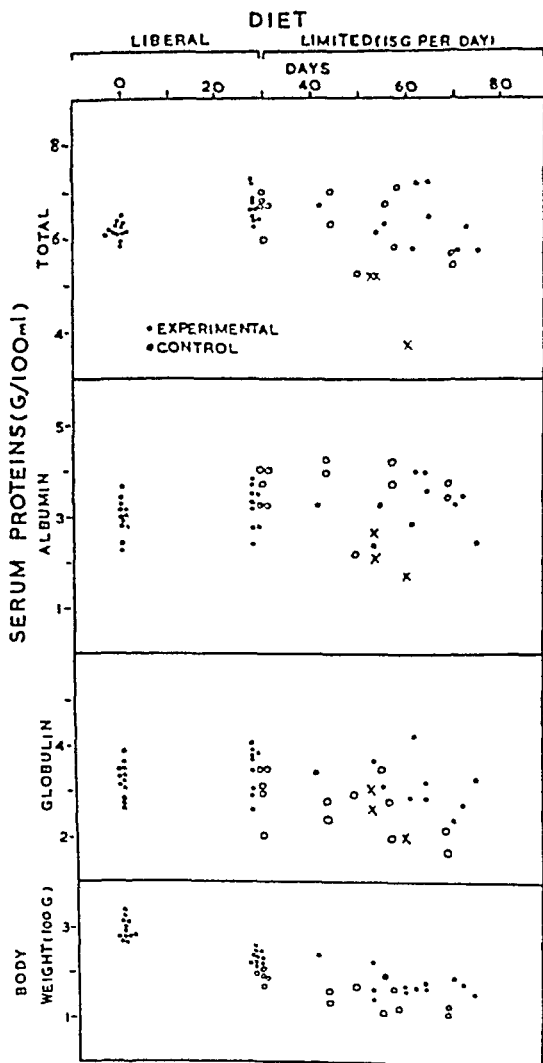
Fig. 7.—Effect of repeated oral administration of dinitro-*o*-cresol on the serum-protein
concentration and body weight in rats.   x = in moribund state.

Liver function tests again gave conflicting results. A false positive
colloidal gold reaction was discovered in one control and in 9 experi-
mental animals.   In 7 of the latter it was present before the
administration of the dinitro-*o*-cresol and in several the test even
became negative after the drug was administered.   The thymol
turbidity test was always negative.

DISCUSSION

The efficiency of the organism in restoring its circulating plasma protein after serious depletion is shown by the convincing observations of Morawitz (1906), Whipple and his collaborators (Smith *et al.*, 1920) and Cutting and Cutter (1935), and by the present experiments. Thus Morawitz found that following plasmapheresis, the normal fasting dog replaces a great deal of the lost plasma protein within a few days, and Smith *et al.* demonstrated some replacement within a few minutes. Restoration goes on at a great pace in the normal rat, for in as short a time as 12 hours after removal of 40 per cent. of the blood, the total plasma protein returns to the original amount (Cutting and Cutter ; my own experiments).

On the other hand, if liver function is disturbed either by decreasing its portal blood supply as with an Eck fistula, by impairing its cell function by poisoning, or by removal of the organ, then, it is claimed, restoration of plasma protein is less successful. We owe to Whipple and his co-workers an elaborate study of this question, employing the Eck fistula technique (Kerr *et al.*, 1918-19 ; Knutti *et al.*, 1937 ; Whipple *et al.*, 1945).

An Eck fistula dog suffers from a reduction in its combined blood-flow through the liver which probably amounts only to 25-35 per cent. of the normal. Such an animal is said to develop up to 20 per cent. of liver atrophy (Whipple and Hooper, 1916-17), with but little fatty degeneration of the hepatic cells and some increase of connective tissue around the bile ducts and portal veins. The latter cannot be of much significance, for Whipple *et al.* (1945) admit that it was very slight in a small group of dogs which survived the procedure for 8 years. These dogs vary from time to time in their capacity to form plasma proteins from standard basal diets, sometimes presenting unusual disturbance of protein metabolism, sometimes behaving in normal fashion. No explanation is offered for these spontaneous variations. The number of dogs employed in Whipple's experiments was small and often there was no great divergency between the experimental and control animals. Whether the variable results can be attributed merely to there being only moderate reduction in the number of functioning elements is difficult to say, but it seems unlikely. Results obtained by Fiessinger and Gothié (1933) are not in agreement with those recorded in the short-term experiments of Whipple, for the French workers found no change in plasma-protein values 2, 6 and 8 hours after production of an Eck fistula in dogs. The fall in albumin 3 and 5 days afterwards they considered to be the result of nutritional upset, as the animal was wasted.

A recent study of plasma-protein regeneration in hepatectomised animals by Mann and his associates (Berryman *et al.*) deserves special attention because of the care taken to eliminate disturbing technical elements. With hepatectomy there is not inconsiderable loss of blood from operative trauma. This may amount to 5-10 per cent. of the

total plasma, albumin loss being 5-10 per cent., fibrinogen 30-100 per cent. and euglobulin 3-100 per cent., but the pseudoglobulin value is usually increased, in some instances rising by as much as 66 per cent. of the original value. All of these variations occur within the first few hours of operation. Subsequent analysis of the blood during the 30 hours of survival in the most favourable experiments shows only minor variations in protein content of the plasma and no major alterations in protein distribution. The blood volume was estimated at intervals, but no great change was detected. Berryman *et al.* conclude that during the period of adjustment immediately following removal of the liver, there may be an addition of pseudoglobulin to the circulating plasma but this is small in amount, rarely exceeding 0·5 g. per kg. body weight. There is a somewhat irregular destruction or loss of fibrinogen and euglobulin but no appreciable change in the plasma albumin.

Withdrawal of $\frac{1}{3}$-$\frac{1}{2}$ of the total blood volume followed by restoration of an equivalent amount of dog red corpuscles in normal saline 10-20 minutes after hepatectomy was practised in some animals. Hæmo-concentration frequently developed with this procedure and had to be allowed for in the subsequent computations of protein values. Some evidence was obtained that an increase of protein occurs during plasmapheresis, amounting to 0·5 to 0·7 g. per 100 c.c. Globulin appeared to be added, but the albumin content remained very close to that expected from withdrawal of that amount of blood and dilution of the remaining blood with saline red corpuscle suspension. During subsequent periods up to 9 hours, only minor changes were discovered in the blood, consisting of slight loss of protein, probably from a small loss of whole blood and subsequent dilution. In no case was any appreciable destruction of albumin or its replacement apparent. No loss of globulin occurred, although in a few experiments a very slight increase of globulin set in several hours after plasmapheresis.

It seems clear from the careful work of Berryman *et al.* that caution must be maintained in the interpretation of complicated and severe operations on the liver and blood, for anæsthesia, surgical trauma, loss of blood from hæmorrhage and the administration of dextrose exert effects on the circulating blood ; moreover, redistribution of the corpuscles and plasma cannot be excluded. These facts make the interpretation of sudden changes in plasma-protein content hazardous, especially at the time of plasmapheresis. An immediate influx of protein into the plasma during plasmapheresis may be due in part to imposed alterations in the circulatory system as well as to withdrawal of protein, since similar changes are found during operation for complete removal of the liver. Here too the course is not clear, for many of the changes in the plasma proteins coincident with total hepatectomy are obviously due to causes other than absence of hepatic tissue, since there is no consistent loss or gain of protein with the progress of time following the operation. It would appear from a

scrutiny of the results of Berryman *et al.* that protein loss under these conditions is too small to be measured with existing methods throughout the animals' 30-hour period of survival. The conclusion thus forced upon us is that plasmapheresis after complete hepatectomy does not induce any regeneration of plasma protein within periods which are adequate for the demonstration of some such regeneration in normal control animals.

The chief support for the theory that the liver is mainly concerned in plasma-protein production comes from the plasmapheresis method of Whipple, Madden and their collaborators (Madden *et al.*, 1937 ; Madden and Whipple, 1940 ; Whipple, 1942 ; Whipple and Madden, 1944). By repeated plasmapheresis and maintenance of a basal diet it is possible to keep healthy dogs at a constant low plasma-protein level, the amount of blood which must be removed during arbitrary time intervals serving as an indication of the regenerative capacity of the animal. On the whole, a healthy liver favours efficient regeneration of plasma proteins, but one cannot escape from the feeling that the process is a highly complicated one, that many factors are concerned and that little direct support comes from this type of investigation for a primary hepatic factor.

Finally, some evidence exists that damaging the liver by certain poisons, *e.g.* chloroform, CCl_4 and phosphorus, impairs the power of the animal to replace its plasma proteins when these are depleted by plasmapheresis (Kerr *et al.* ; Smith *et al.*). It should not be forgotten, however, that poisons are by no means specific in their action on this organ and many tissues may be affected, whereby disturbance of general metabolism can be brought about. I shall refer to this criticism later.

Another type of evidence offered in support of the hepatic origin of plasma proteins originates from procedures whereby the liver function is impaired by poisons, by interference with bile excretion, by reduction of the portal blood supply by Eck fistula, and by partial or complete hepatectomy. It is claimed that side by side with such disturbances there occurs a diminution in plasma-protein concentration which reflects the impairment of the liver cells. Lack of space forbids a review of this evidence, which is often inconclusive and frequently equivocal.

It is advisable at this stage to recall the criticism of Luck (1939), who wrote :—" Indeed few problems in physiology can be in a more confused state than those concerning the origin of the plasma proteins. The field is rich in experiment but, as yet, the role of any single organ has not become clear. *A priori*, we might well regard the liver as an important site of formation, but experiments designed to test this hypothesis have led to conflicting results."

1. It might be expected that complete removal of the liver from the experimental animal would lead to serious disturbance of the plasma-protein content of the circulating blood, if that organ alone

were the site of formation of the plasma protein. It is only fair to
point out that such an argument assumes continual wearing out and
replacement of the protein supply, the life period being short, so
that a significant change in protein concentration would be apparent
after complete hepatectomy. As yet we know little about such a
life period, though modern investigations with isotopes (Schoenheimer,
1946) suggest a dynamic state with active wastage and renewal of
proteins and Schoenheimer *et al.* (1942) hint at a half-life period for
serum proteins of 14 days. Hepatectomy studies disclose a variable
and often unconvincing decrease in plasma protein, with the exception
of fibrinogen ; indeed, the fall in total protein appears to be insignificant
compared with that of prothrombin (Warren and Rhoads, 1939).

Even the modern and sensitive methods of protein investigation
(*e.g.* electrophoresis) have given no proof of marked alteration in
protein patterns (Munro and Avery, 1946). Carefully controlled
experiments which take into consideration the trauma associated
with hepatectomy, partial or complete, suggest that blood loss, fasting
and impairment of general metabolism may be at least as important
as hepatic failure (Fiessinger and Gothié, 1933 ; Weech *et al.*, 1933 ;
Chanutin *et al.*, 1938 ; Chow *et al.*, 1945). My own results with rabbits
are in agreement with these conclusions.

2. Little support comes from Eck fistula experiments and it is
astonishing how much has been assumed from so few data. My
experiments with rats have convinced me that the Eck fistula technique
is unsuitable for the solution of the problem, for little hepatic
disturbance follows this procedure, either after a short or a long interval,
and I have found no significant alteration in the plasma proteins over
fairly long periods in such animals.

3. Preventing bile outflow from the liver has not thrown much
light on the plasma-protein problem. I doubt whether we should
expect it to do so, for I agree with Whipple's criticism that under
the influence of obstructive jaundice there is marked disinclination
on the part of the animal to eat. This has certainly been the case
with my rats during the first week or so after ligation of the common
bile duct. Impaired digestion because of lack of bile in the intestines
may also play a part. It is all the more surprising that despite the
gross structural liver damage invariably found in rats and rabbits
with prolonged common bile duct obstruction, I have been unable
to demonstrate any constant and pronounced alteration in the plasma-
protein concentration. I am compelled to accept an extra-hepatic
origin of the proteins as the result of these experiments.

4. I doubt whether results obtained from the use of so-called
hepatic poisons are of much use in bolstering up the theory. I do
not know of any such poison which is strictly specific in its action,
yet the method is still employed extensively in the study of liver
function.. The effects of poisons are so complicated that conclusions
cannot be drawn about the functions of any one organ, least of all

the liver, even though that organ be seriously damaged. In this criticism I am in close agreement with Williamson and Mann (1923), whose investigations of phosphorus and chloroform poisoning convinced them that often physiological impairment of the liver was not the only factor or even the primary factor in causing death. The process is complex and not only involves such organs as the kidneys, adrenals, heart and central nervous system, but also includes the effect on the general activity of the organism of damage to or destruction of a large amount of body tissue.

5. It is known that even an apparently innocuous procedure such as plasmapheresis may disturb the function of various organs, *e.g.* the kidneys, and upset the electrolyte balance (Barker and Kirk, 1930 ; Darrow *et al.*, 1932). To attribute plasma-protein variation under such circumstances to the liver may be going too far.

6. Most if not all experimental procedures used for placing a strain upon hepatic function are accompanied by metabolic disturbance consequent upon temporary or prolonged fasting. Many organs show a relative loss of proteins during fasting and the blood is no exception (Addis *et al.*, 1936 ; Luck, 1936). Recent work has shown how easily the function of the kidneys, and indeed of the liver, may be put out of gear by circulatory mishaps such as " shock " (Dole *et al.*, 1945-46 ; Phillips *et al.*, 1945-46 ; Long, 1947). Blood loss, infection and nervous factors such as fear add their quota of complications.

7. Modern investigations have brought to light other factors which are concerned in the maintenance of the remarkable plasma-protein equilibrium (Barnett *et al.*, 1932). Thus there is some reason for associating this with an adrenal factor, for after adrenalectomy there may be an increase in the globulin fraction and a diminution of the albumin fraction of the plasma (Hartman *et al.*, 1942). Treatment with adrenal cortical preparations increases plasma-albumin level. Long and his co-workers (Long, 1947) have clearly demonstrated the importance of an adrenotropic hormone synthesised by the cells of the anterior lobe of the pituitary in the catabolism of tissue proteins and the plasma proteins also appear to be under the care of the pituitary. A decrease in plasma albumin and an increase in plasma globulin was recorded in hypophysectomised dogs and rats by Goldberg (1938), Levin and Leathem (1942) and Levin (1942-43). A similar disturbance after thyroidectomy can be prevented by giving thyroxine or cortico-adrenal extract. Such relationships are most likely of the nature of controlling mechanisms and may well influence the main sources of production of the plasma proteins, but I would submit that the evidence for their existence is much the same as that which associates the formation of plasma proteins with the liver and it may turn out that we are dealing with a whole series of interlocked mechanisms which initiate and control the production of plasma proteins, maintaining these in a state of labile dynamic equilibrium with the body proteins.

Where are the plasma proteins formed ?

We are still faced with the mystery of the site of origin of these proteins. Are they formed in one organ or in many tissues ? Is a cellular mechanism necessarily concerned in their formation, or may we hazard the guess that a self-perpetuating device, like that met with in virus proteins, is at work ? Can there be a non-cellular synthesis within the fluids of the body spaces, dependent upon special enzyme systems ? These questions cannot be solved with our present restricted knowledge, though there are some facts which point the way for future investigations.

Because of the speed with which plasma proteins can be replaced after depletion (appreciable amounts may enter the blood within a few minutes), I would postulate a site of storage and production which can rapidly empty its reserves of protein into the blood stream either directly or by way of specially efficient lymphatic channels. The high protein content of liver lymph would seem to indicate that much protein is contributed by the hepatic cells to the fluid leaving the liver. But a similar state of affairs exists within the intestinal wall, the lymph from which is extremely rich in protein, and other proposals have not been wanting from time to time.

(i) The bone marrow may be concerned, either alone or in association with the liver (Müller, 1905 ; Kaznelson and Lorant, 1921 ; Kisch, 1923 ; Levi-Crailsheim, 1923 ; Reymann, 1924 ; Westergren *et al.*, 1931-32 ; Reimann *et al.*, 1934). Most of the evidence for this view applies to fibrinogen. The argument is also based on the well-known increase of protein, especially globulin, in cases of myeloma (Magnus-Levy, 1931-32 ; Reimann, 1932). However, Whipple and Hurwitz (1911) state that the bone marrow is not responsible for fibrinogen production.

(ii) Disintegration of leucocytes liberates plasma proteins. The early work of Schmidt (1892), Pfeiffer (1897) and Mathews (1899-1900) made out a case for fibrinogen production in this way, whilst Moll (1903) and Morawitz (1925) agreed that leucocytic destruction contributed to globulin formation. Downey and Weidenreich (1912) stressed a pinching off of particles containing protein from the protoplasm of plasma cells and lymphocytes, Liebreich (1923) derived proteins from the granules of eosinophils, while Hurwitz and Meyer (1916) thought that absorption of products of disintegrated leucocytes might contribute to the globulin supply. Most supporters of this hypothesis have emphasised the role of the lymphocytes, a view which has recently reappeared in the work of White and Dougherty (1946), who suggest that γ globulin, and possibly β globulin, are formed by these cells. The daily wastage of vast numbers of lymphocytes has long been a puzzle. Their association with globulin production would resolve this mystery, but we must wait until quantitative data are available before accepting this explanation.

(iii) There seems to be little doubt that lymphoid tissue plays an important part in the production of the globulins concerned in antibody activity (McMaster and Hudack, 1935; McMaster and Kidd, 1937; Sabin, 1939; Burnet, 1941; Ehrich, 1946), but whether reticulo-endothelial cells or lymphocytes are chiefly responsible has not yet been decided. Caspersson (1940, cited by Monné, 1948) presented some facts which suggest that both normal blood proteins and antibodies are synthesised by the chromidia of the cells of the reticulo-endothelial system and appear first in the interchromidia.

(iv) Disintegration of red corpuscles (Levi-Crailsheim, 1923; Reymann, 1924) and platelets (Frey, 1933-34) as a source of plasma proteins, especially globulins, has little supporting evidence.

(v) Plasma cells have been suggested as a source of globulins, especially those occurring in pathological conditions and associated with antibodies against various antigens (Ranström, 1946). As early as 1913, Hübschmann suggested that plasma cells can produce antibodies, while Bing and Plum (1937) emphasised the regular occurrence of plasma cells in the blood in association with hyper-globulinæmia, and Bjørneboe and Gormsen (1943) described a close relationship between hyperglobulinæmia and the accumulation of plasma cells in various organs, especially the spleen, in rabbits immunised with polyvalent pneumococcal vaccine. Magnus-Levy (1938) and other investigators supposed that plasma cells in plasma-cell myeloma are responsible for the hyperglobulinæmia so often found in that condition. No direct proof exists for this method of production, although plasma cells are generally held to be modified lymphocytes and we have seen that some evidence has been obtained that lympho-cytes are the source of γ globulins.

(vi) At one time, an origin from the intestinal tract was favoured (Müller, 1905, for discussion) and amongst its supporters was Claude Bernard. Dent and Schilling (1948) found no appreciable changes in amino-acid levels in the portal blood after feeding dogs with homologous plasma protein. This suggests that it was either absorbed intact or re-synthesised in the intestinal wall.

(vii) Cells in general may be capable of synthesising plasma proteins. Amongst these should be considered those of muscle and connective tissue, both of which produce special types of protein in the form of myosin, collagen and certain mucoproteins. Connective tissue cells, and probably though less certainly muscle cells, require plasma proteins for repair; explanted connective tissue cells are capable of synthesising and breaking down blood proteins (Landsteiner and Parker, 1940; Fischer, 1947) by means of enzymes. I would suggest that further investigation of muscle and connective tissue is required before excluding these huge reservoirs of protein, water and salts as likely sources of plasma protein.

All such considerations show that the problem of the origin of plasma proteins is in a highly unsatisfactory state. The matter

demands a fresh attack, freed from prejudices and using strictly quantitative methods, such as those already employed with success for tissue proteins in general by Addis *et al.* (1936) and Luck (1936, 1939). Perhaps the new fields which are being opened up by modern physical investigations will bring the solution.

CONCLUSIONS

The above survey suggests that the present state of our knowledge of the origin of plasma proteins is highly unsatisfactory. The custom of assigning the role of protein production mainly to the liver has apparently sprung from the frequent association of liver diseases with alteration of the plasma-protein level. But the experimental evidence brought forward in support of a hepatic origin is inconclusive and open to serious criticism. The author has failed repeatedly in attempts to induce disturbance of plasma-protein behaviour by subjecting the liver experimentally to severe damage. The facts elicited therefore lead to the conclusion that, in the past, too much emphasis has been placed on the part played by the liver in the elaboration of plasma proteins. The mechanism of synthesis is likely to be far more complicated and extra-hepatic factors probably intervene. The numerous and diverse sites of origin proposed suggest that different plasma proteins may be manufactured in widely separated organs and cells with different mechanisms of synthesis. Evidently much more data on this subject are needed in respect of both normal and pathological plasma proteins.

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MALIGNANT HÆMANGIO-ENDOTHELIOMA
(HÆMANGIOBLASTOMA) OF THE LIVER

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(PLATES XIII AND XIV)

THE following case is presented as an undoubted example of a rare condition which shows several interesting features, both clinical and pathological.

CASE REPORT

Clinical history

A man aged 34, a park attendant, was admitted to St Helier Hospital under the care of Dr W. I. Card, complaining of abdominal pain of one month's duration. He had suffered from epilepsy since the age of twelve and had been under the care of the National Hospital, Queen Sq., London, since 1935. In 1944 he was admitted to St Helier Hospital with concussion sustained by falling while in a " fit." He was bleeding from nose and ears, having probably fractured the base of his skull. He had had no major fit during the previous twelve months. One sister suffered from fits.

The present illness started with knife-like pain in the upper abdomen and swelling of the abdomen generally. There was no previous history of indigestion. He also complained of increasing pallor and weakness of two or three weeks' duration, though he had kept at work until a week before admission. He had observed no loss of weight. Diarrhœa had troubled him for a week before admission.

On examination he was obviously ill, with pallor, a hectic flush and a generally cachectic appearance. His cheeks were scarred. There was slight icterus of the conjunctivæ. He suffered from slight dyspnœa even while lying at rest.

The mouth, throat and gums were normal. One hard cervical lymph-node was palpable. His temperature was 101° F., pulse 110, respirations 20. The heart had a very forcible beat, the apex being in the fifth intercostal space in the nipple line. The first sound was accentuated and a thrill was palpable in the fourth left interspace. Blood pressure was 145/60. Both lung bases were dull to percussion, with crepitations on auscultation.

The abdomen was distended with a massive ascites. On auscultation a remarkable bruit was audible in three areas, one over the right lobe of the liver, one over the left lobe and one in the left lower quadrant of the abdomen in close proximity to a visibly dilated vessel running subcutaneously. The bruit, which was accurately localised to these areas, consisted of a loud rushing, roaring noise, like the sound of the sea on the beach. It was continuous but became accentuated on inspiration. No sign of a collateral circulation other than the vessel mentioned was noted.

The central nervous system showed no abnormality and the discs were normal.

Blood examination showed hæmoglobin 24 per cent. and a normal white-cell count. This anæmia was treated with repeated blood transfusions. X-ray examination of the chest showed some left-ventricular enlargement only. The Wassermann reaction was negative. Bleeding and clotting times were normal. The plasma proteins were 5·5 g. per 100 c.c. Routine urine tests showed no abnormality.

Paracentesis abdominis revealed heavily blood-stained ascitic fluid. It contained numerous mature red cells ; one small clump of endothelial cells was seen after prolonged search ; no evidence of inflammatory exudation was found. Some four pints were drained on each of two occasions. This enabled an enlarged liver to be palpated, together with a mass in the right upper quadrant of the abdomen, apparently in the liver. A diagnosis of some highly malignant vascular tumour causing ascites, probably a malignant hæmangioma, was made.

The patient improved on numerous blood transfusions and paracenteses, stating that he felt well, though his temperature rose daily to 99°-101° F. No change occurred in the bruit. The ascitic fluid varied from a heavily blood-stained to a clear yellow appearance.

Laparotomy was performed by Mr York Mason in the faint hope of finding some remediable condition, but the liver was found to be extensively replaced by masses of growth, bulging the surface of the organ with blue-black cysts and blood-filled cavernous tissue. The abdomen was therefore closed. Despite transfusion, the patient failed to rally after operation and died two days later, with terminal jaundice. Death thus occurred in less than two months from the first appearance of symptoms.

Post-mortem examination

Externally the body was emaciated and slightly jaundiced. The heart was normal, though the aorta was hypoplastic. The lungs were normal, with no sign of secondary deposits.

Several pints of ascitic fluid were present in the peritoneal cavity. The liver was enlarged down to the umbilicus, being in great part replaced by masses of knobbly and cavernous growth : it weighed $10\frac{1}{2}$ lb. On section, but little normal liver tissue could be seen (fig. 1). The portal venous system showed several features of interest. The portal vein itself was dilated, and opposite the entry of the splenic vein the wall was thickened, probably by organisation of a former thrombus. One œsophageal varix was found. The left branch of the portal vein pursued an anomalous course, winding around and under the main trunk, bile duct and hepatic artery to end in a large sinus situated in and largely replacing the left lobe of the liver. A large umbilical vein running in the ligamentum teres opened into this sinus.

The spleen was moderately enlarged, weighing 1 lb. It was engorged, but presented no other abnormality. Several cœliac glands were enlarged and congested and were thought to be the site of secondary deposits. The intestines, pancreas and kidneys showed no abnormality. The brain showed a small area of discolouration about 1 sq. cm. on the under surface of the left temporal lobe but was otherwise normal.

PLATE XIII

HÆMANGIOBLASTOMA OF LIVER
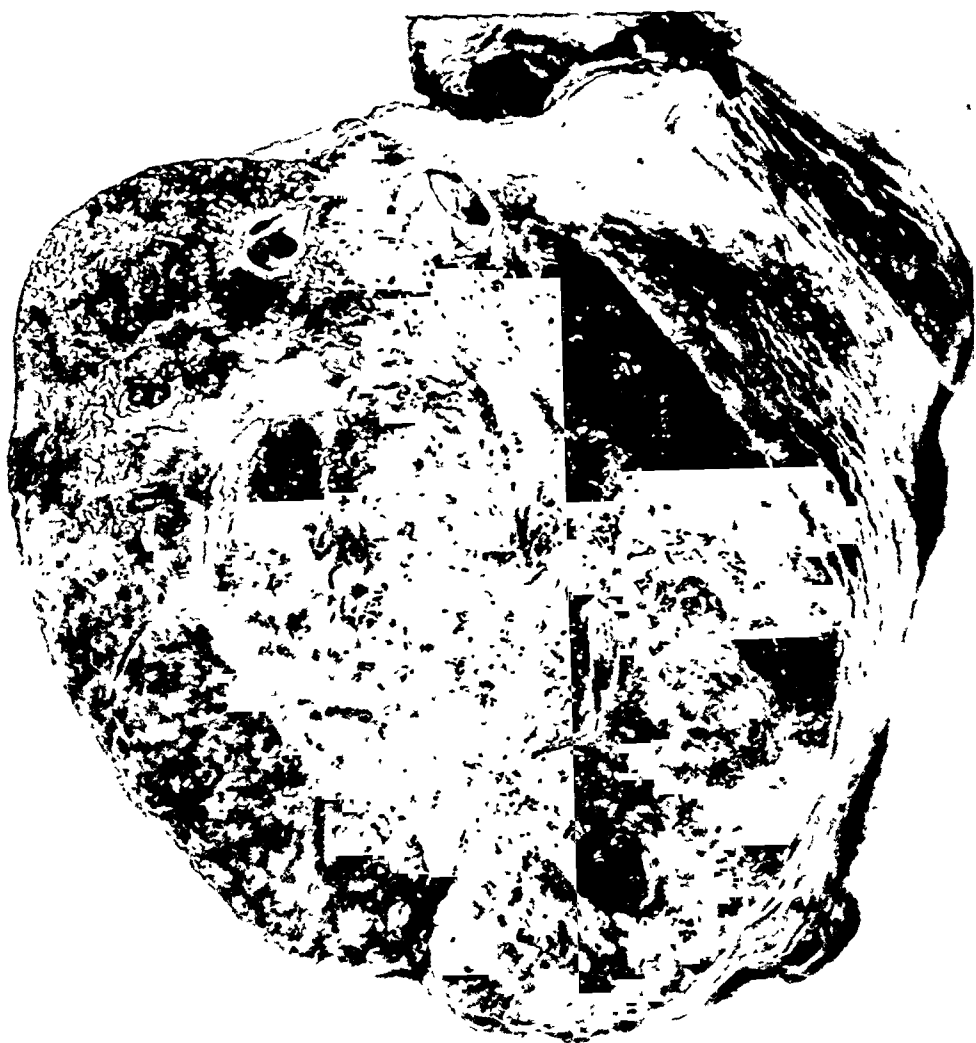
FIG. 1.—Appearance of the greatly enlarged liver on mesial section, showing the hepatic tissue almost completely replaced by new growth. Slightly reduced.

Histological examination

In the liver but few areas of normal hepatic tissue could be found. The appearance varied greatly in different parts. Some areas, for example, showed blood-filled cavernous sinuses lined by many layers of highly malignant-looking, anaplastic endothelial cells which were spreading out into and destroying the surrounding liver tissue. Examination with the high power confirmed this pleomorphic and malignant appearance (fig. 2) and showed the presence of abundant mitotic figures. In close relation to the malignant endothelium and spreading into the surrounding liver tissue were numerous blood islands, apparently in a state of active hæmatopoiesis (fig. 3). In other areas a syncytium was seen spreading into and replacing the surrounding liver tissue, with spaces containing nucleated hæmoglobinised cells of primitive appearance, the whole reminiscent of the earliest stages of embryonic vascular development. Staining with silver brought out the tubular structure underlying the loose meshwork of these sections, the appearance being similar to that depicted by Stout (1943, fig. 2).

In the spleen, general engorgement and vascular proliferation were seen, with some fibrosis, but no sign of malignancy or active hæmatopoiesis. The cœliac lymph nodes showed general enlargement and congestion, with a great excess of blood vessels. None of the primitive endothelial cells were found in the lymph-nodes and it seemed unlikely that metastasis had occurred. The discoloured area in the brain showed signs of an old bruise with pigmentation and gliosis. There was no evidence of hæmangioma.

DISCUSSION

The occurrence of malignant tumours of blood vessels is generally accepted, though the number of reported examples is not large. Stout (1943), in an extensive review, rejects many of the cases in the literature. Willis (1934, p. 151) states that most vascular tumours cannot be differentiated from " hamartomas " or vascular malformations ; the apparent " tumour " being due often to non-neoplastic enlargement of pre-existing vascular tissue. Metastasis also may be only apparent, being really due to multicentric origin, as described by Jaffé (1929) and as in the cases described by Taylor and Moore (1933) and De Navasquez (1936). Again, great care must be taken not to confuse with malignant tumours of vascular origin secondary deposits of a highly vascular or hæmorrhagic nature, such as some cases of sarcoma, or adenocarcinoma with blood in the glandular - acini, and especially chorionepithelioma. It may be noted that Willis (1948, p. 713) regards true malignant tumours of vascular tissue as extreme rarities.

The case now described appears to be a highly malignant hæmangioendothelioma (hæmangioblastoma) of the liver. Geschickter and

Keasbey (1935) describe a similar hepatic type with ascites and no metastases, though in their case the liver was smooth. The case described by Gray (1929) was associated with cirrhosis.

Clinically the rapid enlargement of the liver with blood-stained ascites and the rapid downhill course with cachexia indicated probable malignancy. The remarkable bruit heard over the liver led to the assumption that this was vascular in origin, while the character of the hum with its respiratory accentuation pointed to its close connection with the venous system. A clinical diagnosis of hæmangioblastoma was thus made and was confirmed at laparotomy.

The detection of such a bruit does not seem to have been described hitherto in this connection and is a sign worth seeking where tumours of this type may be in question. The case described by Smith and Horton (1939) had the well-known bruit of an arterio-venous aneurysm, and was in fact an example of that condition : it should not have been designated hæmangioma.

Both the macroscopic and the microscopic appearances indicate the malignant nature of the present tumour, though opinions differed as to whether there were secondary deposits in the cœliac glands. The highly pleomorphic character of the malignant endothelium is worth noting, since it might possibly be regarded as an expression of the multipotency of these primitive cells. Willis (1948, p. 713) speaks of these tumours as mesenchymomas of mainly vasoformative character, thus stressing their possible multipotency. Of particular interest in the present case is the occurrence of mature and immature red cells, both as islands in the midst of the neoplastic tissue and lining the vascular channels of the tumour and the adjacent liver, such foci of hæmatopoiesis being widespread in some parts of the growth. Their close relation to the tumour, the fact that no primitive cells were seen in blood films during life, and the absence of such foci from lymph nodes and spleen led to the conclusion that this was an example of an additional mode of development open to these primitive endothelial cells. Though Shaw (1928) spoke of the possibility of such a line of differentiation I have been unable to trace any description in the literature of a case in which this had in fact occurred.

The brain was examined with particular care but no sign of a vascular tumour was found. The old scar was thought to be a residuum from the injury for which he was admitted in 1944.

There was no history of the use of thorotrast in previous investigations of his central nervous system. Burrows (1937-38) described nævus-like lesions in the liver and spleen of rabbits after the injection of this substance and MacMahon *et al.* (1947) have reported what may have been a clinical example.

The congenital anomalies found in the portal system are noteworthy and lend support to the idea that these tumours are themselves of congenital origin, being inborn defects of the vasoformative tissues.

PLATE XIV

HÆMANGIOBLASTOMA OF LIVER
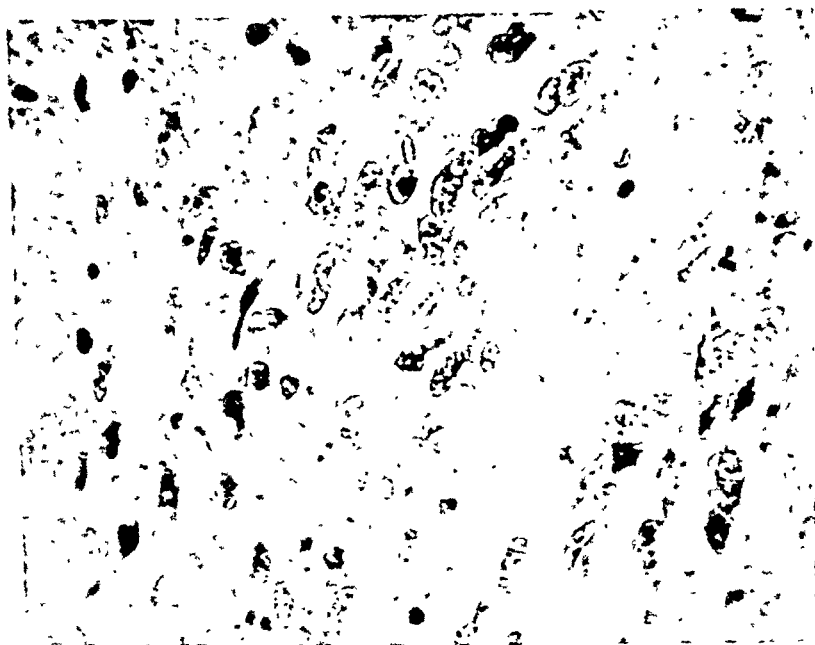
FIG. 2.—High-power view of an anaplastic area of the hepatic tumour, showing cellular and nuclear heteromorphism.   Hæmalum and eosin.   × 420.
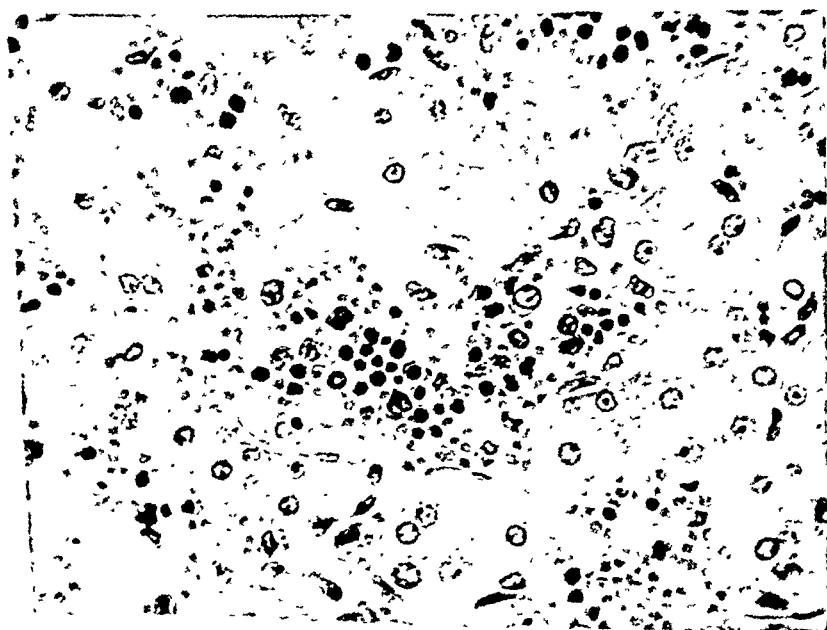
FIG. 3.—Liver showing foci of active hæmatopoiesis.   Hæmalum and eosin.   × 420.

If so, the malignancy was presumably a secondary occurrence on the basis of the underlying developmental error.

SUMMARY

1. A rapidly fatal case of malignant hæmangio-endothelioma (hæmangioblastoma) of the liver occurring in a young man is reported.

2. A remarkable bruit was heard over the liver which, taken with the other signs, enabled a correct pre-operative diagnosis to be made.

3. The multipotency of the primitive endothelial cells composing the tumour is pointed out, there being clear evidence of hæmatopoiesis. This does not appear to have been noted before.

4. The theory that these hæmangiomata are primarily developmental malformations or hamartomas is supported in this instance by the finding of obvious developmental anomalies in the vascular arrangements of the liver.

I should like to thank Dr R. A. Willis for his help and encouragement in the investigation of this case and Dr W. G. Millar for fig. 1.

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STUDIES ON THE PATHOGENESIS OF A CASE OF INFLUENZA-A PNEUMONIA OF THREE DAYS' DURATION

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(PLATES XV-XVIII)

IN a former publication, Straub and Mulder (1948) described epithelial lesions in the respiratory tract in human influenza resembling the pictures experimentally produced in the air passages of mice and ferrets. In none of the cases described, however, was it possible to study early epithelial damage, as the lesions found were already characterised by complete loss of ciliated and goblet cells.

In February 1947 we were in a position to analyse a case of influenzal pneumonia which terminated lethally in three days owing to secondary bacterial tracheo-bronchitis and bronchopneumonia caused by hæmolytic *Staphylococcus aureus*. In this case it was possible to gain an insight into the histology of the early and probably influenzal epithelial lesions of the respiratory tract in the human disease.

Case report

Clinical history

K., a male aged 27, became acutely ill on the evening of 1st February 1947, with rigor, sore throat and severe headache. On 2nd February the temperature was 102° F. and the patient began to cough and expectorate hæmorrhagic sputum. On 3rd February he became unconscious and next day was admitted to the Medical Clinic of the University Hospital. On examination he was found to be in a highly toxic state, with severe cyanosis of the face and extremities. Temperature 104°, pulse 120, blood pressure 100-70, respirations 50. Meningeal symptoms. No tonsillitis. Pneumonic consolidation of all three lobes of the right lung and focal consolidation of the left lower lobe.

Laboratory examinations. Oxygen saturation of arterial blood 80 vol. per cent. Leucocytes 1500/c.mm., eosinophils 0 ; very severe degeneration of neutrophils. Urine contained a trace of albumin and many casts. Spinal fluid under increased pressure, cells 85/c.mm., sterile. Patient was no longer able to expectorate. Blood culture yielded three colonies of hæmolytic *Staphylococcus aureus* per ml. Lung puncture of the right lower lobe yielded pus with staphylococci, and on

culture a pure growth of hæmolytic *Staphylococcus aureus* was obtained. Therapy consisted of penicillin 800,000 units per 24 hours, shock treatment and oxygen administration. The patient died on 4th February at 10 p.m.

This rapid death from staphylococcal pneumonia of a young adult made us think of influenza (taking into account the fact that there was a slight degree of influenza in Holland in January and February 1947), and an autopsy was performed by Dr P. H. M. Schillings two hours after death.

Post-mortem examination

Trachea and lungs were removed *in toto* and, as indicated by Straub and Mulder (1948), fixed intact in formalin, portions being taken for microscopic examination after a week. Before fixation, small portions were carefully taken of the trachea and all lobes of both lungs for bacteriological and virological examination.

The most important lesions were found in the respiratory tract. The trachea and right bronchus were of a deep red colour and covered with adherent greenish-yellow flakes of fibrin. The right lung was voluminous and of firm consistence. The pleura was covered with fibrin. The cut surface of the lung was deep red, with numerous small abscesses scattered over it. The left lung was but little changed : only in the basal parts were there some bluish-red spots of consolidation.

The other organs did not show any special lesions. The liver appeared œdematous and showed peri-capillary œdema microscopically. The spleen was not enlarged and the pulp was not softened.

Bacteriological examination

From the mucosa of the trachea a pure culture of hæmolytic *Staphylococcus aureus* was obtained. Gram-stained films of pus from the abscesses in the right lung showed only staphylococci and again a pure culture of the same organism was obtained. Hæmolysis by all the isolated strains was weak. They were coagulase-positive and sensitive to 0·04 units of penicillin per ml.

Virological examination *

The material for investigation was stored for 16 hours at −130° C. The mucosa of the trachea was ground in a mixture of equal parts of saline broth, with 100 units of penicillin per ml., and inoculated intranasally into a ferret. The remainder was stored at −130° C. Owing to circumstances amniotic inoculation into the chick embryo could not be performed until seven months afterwards. Ground-up tissue from the lobes of both lungs was mixed together and, after the addition of penicillin, inoculated intranasally in a ferret. Rigid isolation of the animals was possible.

The ferret inoculated with emulsion of the trachea showed fever after three days, with severe rhinitis, and a strain of influenza virus was isolated in passage ferrets and seven months later from the turbinate of this first ferret (stored at −130°) in the amnion of the chick embryo. A second ferret, inoculated with emulsion

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* Details of this examination have already been published (Antonie van Leeuwenhoek, 1948, xiv, 184).

of the trachea, also became ill, and after two weeks the blood serum contained immune bodies against the isolated strain from the first animal. Seven months later we failed to isolate the strain of the original material in the amnion of the chick embryo. The isolated strain was an A-strain and, even after six passages through ferrets, it was much more difficult to adapt to mice than the Dutch A-strains from the 1939 and 1941 epidemics (Mulder (1940), van Bruggen and co-workers (1947)). As our stock of laboratory strains which were used in February and March 1947 (PR$_8$, Lee, WS, Gatenby, A (1941 N)), showed a high mouse virulence, a laboratory contamination of the material appeared very improbable. Moreover in the cross hæmagglutination-inhibition tests with the above strains in January 1948 it appeared that there existed but slight serological relationship with them and with the A-strains Talmey, Christie and swine-influenza (Shope), (kindly sent to us by Dr C. H. Andrewes). Classification as an A-strain therefore could only be established with certainty by means of cross complement fixation tests with ferret sera and by the Hirst technique with a human serum-pair of another case of influenza A.*

The symptoms of the ferret inoculated with lung emulsion were not typical for influenza. After five days there occurred a fever peak (104° F.) without rhinitis. No passages were performed. The post-infectious serum of this ferret contained immune bodies against the isolated strain (Hirst technique and mouse-protection test). After six months the same lung material (having been stored at −130°) was again inoculated in ferrets and passed, but then we could not isolate a virus strain in ferrets, or, from there, in the chick embryo. Nor did the sera of passage ferrets show any immune titre against the strain. Direct inoculation of the ground lung suspension in the chick embryo also remained negative after seven months' cold-storage. We have therefore reason for assuming that, in contradistinction to what is generally known of A-strains, the " lung-virus " has died out in the original material after six months at −130°. Possibly this is also true for the virus in the mucosa of the trachea. It is known that some B-strains in original garglings may also die off comparatively rapidly after being thawed from low temperatures (Hirst, 1947). Francis *et al.* (1947) also failed to isolate A-strains in the chick embryo in March 1947, though they easily succeeded in the ferret.

With the Hirst technique the titre of the patients' serum against PR$_8$ (influenza A) was 24, against Lee (influenza B) 96, and against the isolated strain <12.

Pathological histology of the respiratory tract

Technique. Formalin fixation ; celloidin embedding ; hæmatoxylin and eosin and muci-carmine staining.

Tonsils. These did not show any lesions.

Larynx. The epithelium of the larynx was examined in an area immediately below the glottis, where there is a covering of stratified squamous epithelium. The posterior wall shows defects in the epithelium, with severe fibrino-purulent inflammation. Bordering on the epithelial lesions, the stratified epithelium shows a few rows of cells displaying parakeratosis, with numerous mitoses in the basal-cell layers.

Trachea. All sections show a more or less extensive fibrino-purulent inflammation of the mucosa, which continues exclusively

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* Afterwards the strain proved serologically related to 1947 strains from Sweden (Gg), England (Barratt) and America (Rhodes). All the sera used were freed from non-specific inhibitor by a filtrate of *Vibrio choleræ.*

into the right main bronchus and its branches. The left bronchial tree is entirely free from inflammatory changes and the epithelium is everywhere normal. At the bifurcation of the trachea the normal tracheal epithelium of the left half passes into severely damaged epithelium of the right. In the region of the bifurcation, a portion of the trachea was examined in serial section. Fig. 1 shows one of
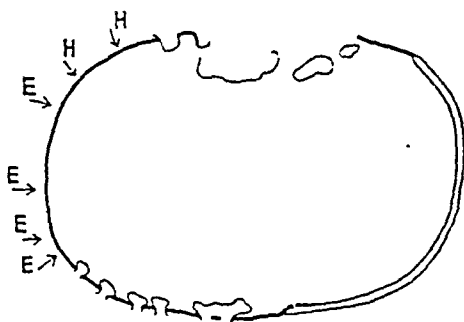
Fig. 1.—Cross section of bifurcation of trachea seen from below (×2). Double contour = zone of normal epithelium on left side (see fig. 2). Dotted line = transitional zone between normal and pathological epithelium in anterior wall (see fig. 3). Single line = zone of pathological epithelium (see figs. 4-10), interrupted by mushroom-shaped fibrino-purulent clots (see fig. 11). H = zone of hæmorrhagic mucosa. E = zone of purulent infiltration of mucosa.

these sections diagrammatically, together with the character of lesions found in it. Fig. 2 shows a photomicrograph of the normal epithelium of the left half of the bifurcation of the trachea.

Pathological changes in the epithelium in the right half of the bifurcation of the trachea. Very interesting pictures were seen in the epithelial zone at the junction of the left and right halves on the anterior wall of the bifurcation. The normal epithelium loses its orderly palisade-like structure, the nuclei are more rounded and their long axes are often not perpendicular to the surface. Nuclei may even be seen in the horizontal position. Here and there the cilia are indistinct and sometimes they disappear entirely. The goblet cells become indistinct or they may swell considerably. There is intercellular œdema. Sometimes cells are pressed out of position by swelling resulting from what is probably local necrobiosis (fig. 3). Mitoses are few in this zone. Infiltration with lymphocytes is no greater than in the normal epithelium of the left half.

Beyond this transitional zone the epithelium shows more advanced pathological changes which are polymorphous in character, hardly any two areas being quite alike. In a few places only does the epithelium display the same picture as that described above, but disintegration of the individual cells is greater (fig. 4). There are also, however, places where we see a different type of lesion—where the epithelium is only about half the normal height. In these areas the still recognisable cilia on the surface show that this epithelium is

PATHOGENESIS OF INFLUENZAL PNEUMONIA

FIG. 2.—Normal ciliated respiratory epithelium from left side of bifurcation of trachea with some lymphocytes between the epithelial cells. Regular nuclear arrangement, the basal and other nuclei having their long axes perpendicular to the basement membrane. In the mucosa no hyperæmia and little mononuclear infiltrate. ×300.

FIG 3.—Slight degeneration of tracheal epithelium at bifurcation. On the left a swollen goblet cell: in the centre " degeneration " with displacement of surrounding cells. Beginning intercellular œdema: cilia and basement membrane unimpaired: hyperæmia of mucosa. No purulent exudate. ×300.
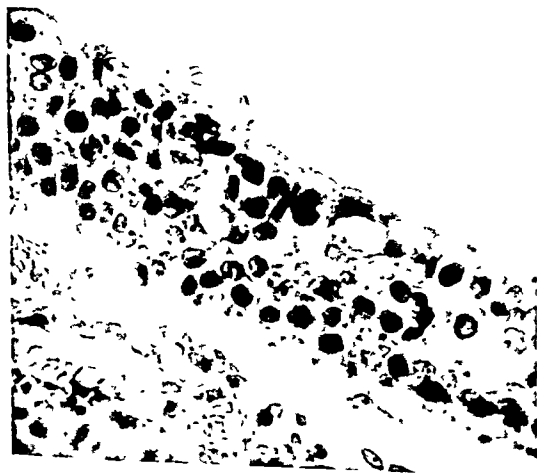
FIG. 4.—Greater loss of structure of tracheal epithelium at bifurcation. Irregular position and degeneration of nuclei : swelling of protoplasm : distinct intercellular œdema. Swelling of basement membrane with œdema under basal layer of epithelial cells. Hyperæmia and hæmorrhage in mucosa. ×300.

PLATE XVI

PATHOGENESIS OF INFLUENZAL PNEUMONIA
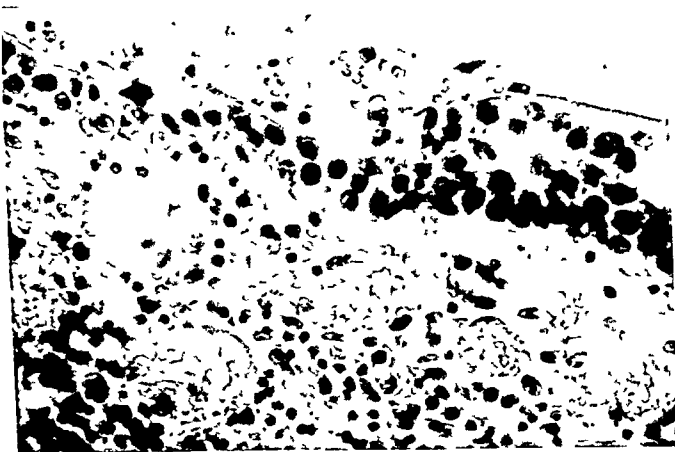
FIG. 5.—Partial desqua-
mation of necrobiotic
epithelium at bifurca-
tion of trachea. Hæmor-
rhage between basement
membrane and epithe-
lium : hyperæmia and
mononuclear infiltration
of mucosa. × 300.

FIG 6.—Considerable necrobiosis of
respiratory epithelium at bifurcation
of trachea, only the basal-cell layer
remaining. the nuclei of which are
round or oval and have their long
axes parallel with the surface of the
epithelium Mitosis in vertical direc-
tion Hæmorrhage under the epi-
thelium. × 300.
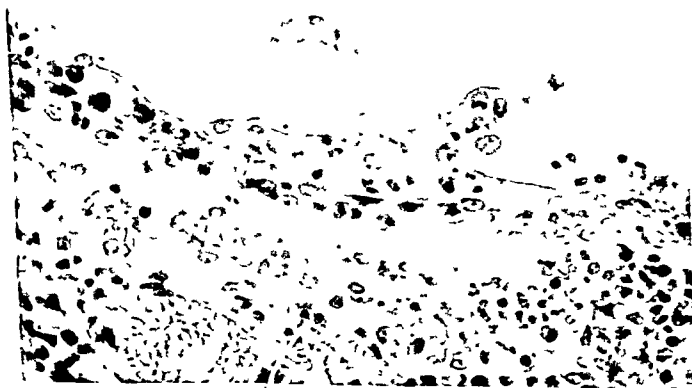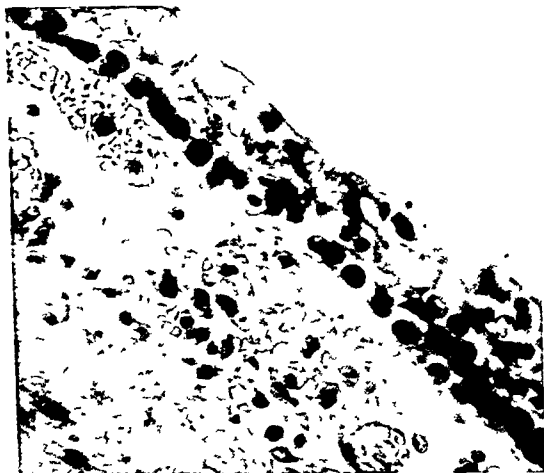
FIG. 7. — Necrobiosis
and desquamation of
epithelial cells at bi-
furcation of trachea.
Mitosis in basal-cell
layer. Nuclei with
long axes parallel
with surface To
right, acute inflam-
mation in mucosa
× 300.

indeed lower, and that it is not desquamated. Goblet cells can hardly be recognised. The nuclei of the cells often have an oblique or sometimes a horizontal position. This epithelium may gradually merge into normal epithelium, as may be seen, for example, in the ducts of the tracheal glands. Elsewhere all the cells become rounded, with intercellular vacuoles but without desquamation. Here we may find a few mitoses in the basal-cell layer. More serious structural loss is caused by desquamation of epithelial cells, so that only two rows of cells remain, rarely only one row, with round or horizontally situated oval nuclei (fig. 5). Here especially, numerous mitoses may be seen, mostly in the basal layer but also in the second layer of cells (figs. 6 and 7). Cell division mostly takes place in a horizontal but sometimes in a vertical direction. Transitional phases with one layer of cells surmounted by a layer which is partly necrotic and without cell structure and partly desquamating are numerous. It is rare to find only one row of basal cells remaining, and then only over short distances. Total disappearance of the basal-cell layer in the absence of fibrino-purulent inflammation was found on one occasion only and may have been an artefact (fig. 8). On top of the basal layer of cells we sometimes see a second layer of flat cells with pyknotic nuclei (fig. 9). It is in the neighbourhood of fibrino-purulent inflammation with defects in the epithelium that these single and double layers are most often seen. In these places mitoses are less frequent. Nowhere do we find pictures suggesting regeneration to three or more layers of cells.

An acute fibrino-purulent (bacterial) inflammation has completely destroyed the epithelium for short distances, so that small defects have arisen. Fibrinous clots of different dimensions and often mushroom-shaped, with intermingled red cells, white cells and masses of staphylococci, protrude through these epithelial defects into the lumen of the trachea (fig. 11). Where there is acute mucosal inflammation without defects, the epithelium may show infiltration with leucocytes, but only for short distances. In general, it is in these areas that desquamation of the pathological epithelium is most considerable.

Basement membrane and mucosa. Slight or more extensive hæmorrhages frequently occur between the basement membrane and the epithelium (figs. 5 and 6), sometimes lifting the epithelium like little hills. It is over these hæmorrhages that desquamation is most pronounced. Elsewhere one may observe œdema between the basal epithelial layer and the basement membrane (fig. 7). Where there is no acute bacterial inflammation, the mucosa shows considerable hyperæmia and dense infiltration with mononuclear cells.

Glands. Epithelial lesions may be found far up the ducts of glands (fig. 10), but mostly for a short distance only near the orifice, or they may be completely lacking. The glandular parenchyma itself does not show any lesion.

Bronchi.   The main bronchus of the right lung and its branches show an almost diffuse fibrino-purulent inflammation of the mucosa. Only small intervening portions of epithelium are available for study. Here also we find both degeneration and regeneration (mitosis), as in the case of the tracheal epithelium.   A small area in one of the segmental bronchi of the right lower lobe, however, shows, on the inner surface, perfectly normal bronchial epithelium, interrupted only in a few places by fibrino-purulent exudate in which staphylococci occur.   Sometimes one sees perfectly normal ciliated epithelium at the margins of a defect.   Elsewhere the epithelium bordering on a defect shows loss of normal structure, but for very short distances only (fig. 12).   Even infiltration with partly pyknotic leucocytes does not cause the epithelium to change greatly, and only for very short distances.   Occasionally epithelial lesions occur at the site of these defects, as in the right half of the trachea.   Lower down we again find fibrino-purulent bronchitis, extending down to the bronchioles.

Small bronchioles.   The small bronchioles of the right lung show low cylindrical epithelium with oval upright nuclei ; nowhere are lesions seen like those described above in the trachea and larger bronchi.   The epithelium remains normal even where it borders on ulcers caused by the acute purulent inflammation.

Lungs.   The right lung shows the well-known picture of confluent hæmorrhagic and purulent focal pneumonia, with numerous abscesses in which lie large accumulations of staphylococci.   The small foci in the left lower lobe show a similar picture.   As the left bronchus and its branches do not display inflammatory changes, these foci may possibly have originated by aspiration from the fibrino-purulent laryngo-tracheitis.   A hæmatogenous origin is also possible.

Interpretation of the pathogenesis of the epithelial lesions and the bacterial bronchopneumonia

The pathological changes in the epithelium described above raise again the question whether they must be looked upon as an influenza virus lesion of the cells or due to the action of staphylococcus toxin. Extensive experimental investigations on staphylococcal infection of the bronchi and lungs of animals is still lacking and indications from human pathology cannot be had, because in the older literature the presence or absence of influenza virus was not adequately studied in cases of staphylococcal bronchopneumonia.

The fact that in our case extensive staphylococcus infection was often found in the area showing considerable epithelial changes (fig. 1) is not in itself a counter-argument to the assumption that these changes are caused by the influenza virus.   We may argue that precisely in the areas of the primary epithelial viral lesion will the staphylococcal infection be most likely to gain entry into the tissues of the mucosa. For the assumption of an epithelial viral lesion it is also important

PATHOGENESIS OF INFLUENZAL PNEUMONIA
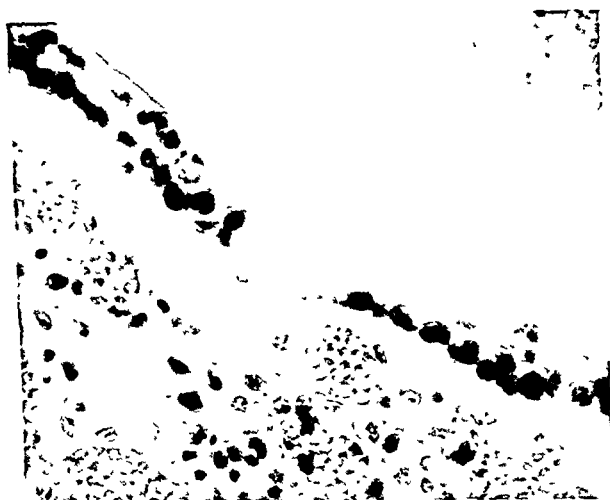
FIG. 8.—Mucosa from bifurcation of trachea. Epithelium consists of one or two layers of cells only. In centre, a defect. To right, only one row of cells with round nuclei. To left, a mitosis. Severe hyperæmia and hæmorrhage in the mucosa: no purulent exudate. ×300.

FIG. 9.—Mucosa from bifurcation of trachea. Epithelium shows one or two rows of nuclei only: the upper row of cells is often considerably flattened. Severe hyperæmia of mucosa. ×300.
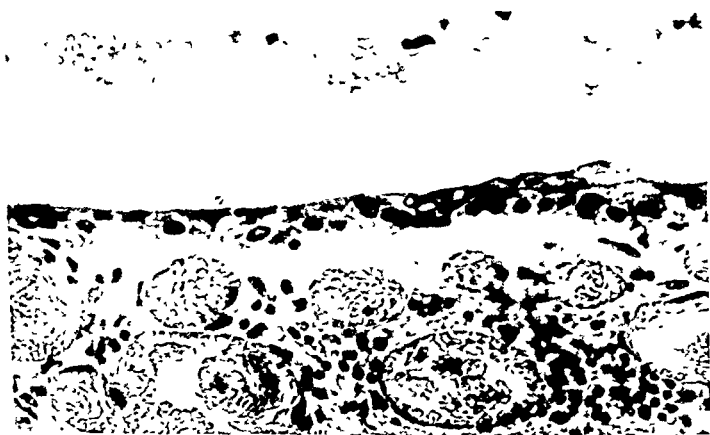
FIG. 10.—Mucosa from bifurcation of trachea. The epithelium of the duct in the centre of the field shows similar changes to those in trachea. To the left of the field shown there was an epithelial defect with fibrino-purulent exudate, some of which is visible in the lumen of the trachea. ×75.
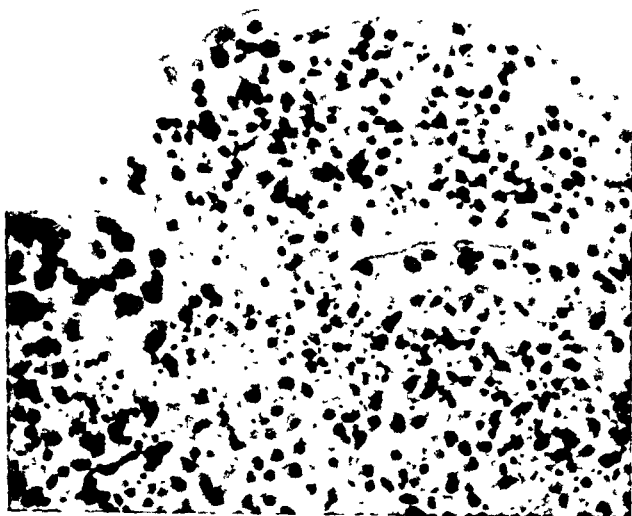
FIG. 11.—Mucosa from bifurcation of trachea. Epithelial defect, with protruding mushroom-shaped fibrino-purulent clot. The surrounding epithelium shows pathological changes. ×300.

FIG. 12.—Segment of lower-lobe branch of right main bronchus next to an epithelial defect with acute fibrino-purulent inflammation. On the left, the epithelium shows a normal aspect. On the right, cellular purulent exudate is present in the mucosa and between the respiratory epithelial cells. ×300.

that in one area of distribution of the right segmental bronchi of the lower lobe staphylococcal inflammation is found without the epithelium showing the characteristic degenerative lesions found in the trachea and right main bronchus.

We believe also that the fact of the basal layer of cells of the respiratory epithelium still remaining pleads against a purely staphylococcal toxic lesion, in which case it would be difficult to understand that it would spare a certain type of cell, whereas this fact could be plausibly explained on a virus hypothesis. The freedom of the epithelium of the small bronchioles from the type of change mentioned above supports the idea that in this case the lung foci may have been caused chiefly by aspiration of cocci from the higher tracheal and bronchial foci of inflammation. As virus has been demonstrated in the ground-up pieces of lung tissue, it is possible that it has acted also as pacemaker for the lung process. With regard to the demonstration of influenza virus in the lung, however, we must also take simple aspiration (*ante mortem* ?) from the air passages into consideration. At any rate the analysis of this case gives support to the idea that a patient may die from slight and in itself innocuous influenza virus tracheo-bronchitis, if, owing to accidental secondary infection with a pathogenic staphylococcus, bacterial foci are formed in the air-passages, and, by aspiration, cause extensive pneumonic inflammation.

SUMMARY

A case is described of influenza-A of three days' duration (February 1947), terminating in tracheo-bronchitis and bronchopneumonia due to the *Staphylococcus aureus*, where, in the trachea and bronchi of the right lung, besides acute fibrino-purulent inflammation, very peculiar degenerative and regenerative epithelial changes were found, with severe hyperæmia and mononuclear infiltration of the mucosa. It is probable that these epithelial lesions were due to the influenza virus and not to staphylococcal toxin. The probable pathogenesis of the process is discussed.

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In the present investigation, late-lactose-fermenting coliform bacilli were collected with the hope of finding a common antigen among the group of paracolon bacilli by which these organisms might be readily identified in clinical pathological practice.

<center>MATERIAL AND METHODS</center>

<center>*Source of strains*</center>

1. One source of paracolon bacilli was the fæcal flora of patients in an epidemic of infantile diarrhœa which failed to yield any well-established bacterial pathogen. Children mainly under two years of age were affected and 12 of the 15 cases were fatal. Thirty-three strains of late-lactose fermenters and non-lactose fermenters were isolated ; 8 paracolon strains were isolated from 47 specimens of stools obtained from 39 patients, and 25 were collected from 95 specimens of fæces from the nursing staff.

2. A collection of 63 late-lactose fermenters from routine cultures consisted of 35 fæcal and 28 urinary strains. The urinary strains were originally isolated in pure culture on routine McConkey plates.

3. Five type species were obtained from the National Collection of Type Cultures : N.C.T.C. 1748, 4647, 5253, 708, 4702.

For the purposes of this survey, paracolon bacilli are defined as Gram-negative, non-sporing, aerobic bacilli of the coliform type which fail to ferment lactose, or ferment it only feebly after 18 hours at 37° C. Gelatin is not liquified even after 3 weeks at 37° C. Motility, reaction to litmus and acetylmethyl-carbinol and indole production are variable.

Fermentation of the following carbohydrates was tested : lactose, sucrose, salicin, dulcitol, glucose, mannitol, maltose, arabinose, rhamnose and xylose. Four sugars—lactose, sucrose, salicin and dulcitol—were selected for the purpose of classification, and table I gives the distribution of 99 paracolon strains according

<center>TABLE I</center>

<center>*Distribution of 99 paracolon strains according to their fermentation of four sugars*</center>

Sucrose +								Sucrose −							
Dulcitol +				Dulcitol −				Dulcitol +				Dulcitol −			
Salicin +		Salicin −		Salicin +		Salicin −		Salicin +		Salicin −		Salicin +		Salicin −	
Lactose +	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
14	5	17	0	1	0	1	0	25	10	4	4	9	2	5	2
(5)*		(8)		(1)				(4)	(5)	(1)		(1)			(1)
a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p
7973†		7924						1111	1748			7893			
8144		8179						1136							
								4308							

* The figures in brackets denote the number of urinary strains in each group. The letters *a–p* indicate the biochemical group.

† Nine key strains from which sera were prepared.

to their fermentation of these four sugars among 16 groups, *a–p*. Sera were prepared against 9 key strains from the larger groups : group *a* (strains 7973 and 8144), group *c* (strains 7924 and 8179), group *i* (strains 1111, 1136 and 4308),

group *j* (strain 1748) and group *m* (strain 7893). All these were recently isolated fæcal strains except 4308, which was isolated from a patient with acute cystitis, and strain 1748, which was received by the N.C.T.C. in 1924 from Castellani as *Bacterium columbense.* ·

Sera and suspensions

This classification, though logical, is artificial, because in some groups there are either no strains or only a few. Accordingly key strains were chosen for serum production only from groups containing a substantial number of strains.

By preparing a number of sera from strains in different biochemical groups, I hoped to determine an association between antigenic and biochemical type. All sera were made against suspensions killed by exposure to 60° C. for 1 hour. Rabbits received intravenous injections twice a week. The first dose was 10^8 organisms and subsequent doses were increased at each injection until a dose of 10^9 was reached.

O suspensions were made by washing the 18-hour agar growth from Roux bottles and steaming the organisms at 100° C. for two hours by the method of Kauffmann (1935-36). The centrifuged deposit was diluted in 0·25 per cent. formol-saline to make a suspension of opacity equivalent to 60×10^9 *Bact. coli* per ml. H suspensions were prepared by growing the strains in 20 ml. nutrient broth at 37° C. for 18 hours and a further 24 hours at room temperature. The organisms were killed by adding formol to make a final concentration of 0·25 per cent. The suspensions were kept at 0° C. for 48 hours, centrifuged and re-suspended in 5 ml. of 0·25 per cent. formol-saline. From these stock suspensions, dilutions were prepared as required.

Each key strain was tested against the nine test sera. If antigens were shared by organisms of the same or different groups, the relationship between the pair of strains was measured by mirror-absorption tests. The minimal absorbing dose (M.A.D.) of each suspension for each of the two sera was determined, and after absorption of an arbitrarily selected dilution of serum with 2 M.A.D. for 2 hours at 37° C. and 20 hours at 0° C. the absorbed serum was tested against agglutinable suspensions containing 10^9 organisms per ml.

The serological relationships of key strains

Only one pair, 7973 and 4308, was found to have a common O antigen. Whereas both absorbed all the agglutinins from serum 7973, absorption of 4308 by 7973 suspension reduced the titre only 8-fold, suggesting that though 7973 and 4308 shared a major antigen, there was a minor antigen peculiar to 4308. None of the remaining 7 strains shared somatic antigens. The main O antigens of the 9 strains were designated by the Roman numerals I-IX (table II). On this basis strain 7973 has IX and 4308 has IX and V.

Each of the following pairs of strains shared an H antigen, but the first in each pair had an antigen, either H or O, not possessed by the second: 1748 and 4308; 1111 and 1136; 8144 and 1748; 1748 and 8144; 8144 and 4308; 4308 and 8144. There was no detectable relationship between any other of the remaining combinations of pairs. The flagellar antigens were designated by the Arabic numerals 1-7. In table II it has been deduced that 8144 = 1, 5; 1748 = 2, 5; 4308 = 5; 1136 = 4; 1111 = 4, 6. ·

Serological relationships of 90 strains tested by the 9 key sera

Flagellated and non-flagellated suspensions were prepared from each of the 90 paracolon strains. Their agglutinability and serological relationships to the key strains are shown in table II. If a strain

TABLE II

Distribution of O and H antigens in 9 key strains and the agglutinability of 90 paracolons by the key antisera

Serum	O antibody	Titre	Number of strains agglutinated at		H antibody	Titre	Number of strains agglutinated at	
			>1 in 8	1 in 16–1 in 64			>1 in 8	1 in 16–1 in 64
8144	I	1280	2	3	1, 5	2560	9	1
1748	II	320	4	0	2, 5	2560	6	0
8179	III	640	3	0	...	...	...	...
1136	IV	2560	2	2	4	2560	3	0
4308	V, IX	2560	9	0	5	640	7	0
1111	VI	2560	13	9	6, 4	2560	16	4
7893	VII	2560	5	1	...	...	...	...
7924	VIII	5210	2	0	7	2560	1	0
7973	IX	2560	4	0	...	...	...	...
	Totals		44 + 15 = 59 (65·5 per cent.)				42 + 5 = 47 (52·2 per cent.)	

was found to contain two or more somatic antigens it was grouped with the test serum with which it gave the highest titre. Similar tests were made of strains having no somatic antigen corresponding to the antibodies in the 9 test sera, but having one of the flagellar antigens.

Table III records the 75 (83·3 per cent.) agglutinable strains, showing the multiplicity of antigens in body and flagella as compared with the key strain. Only 3 strains appeared to be similar to the key organisms. Two strains, 2109a and 5168 resembled 7893, and H and O suspensions of 1014 were agglutinated to titre by the appropriate 1111 sera. Immune serum was prepared against 1014 and reciprocal absorption tests with this strain and 1111 proved that these organisms were antigenically identical. Immune sera were not prepared against the other strains.

Fifteen strains (16·7 per cent.) were not agglutinable ; fifty-nine (65·5 per cent.) could be grouped by their somatic antigen, 74·5 per cent. being agglutinated to a titre not less than one-eighth of the standard agglutination. Eleven strains (18·6 per cent.) shared a somatic component with more than one type strain. There was much overlapping in agglutination with flagellar antisera, and 76·6 per cent. showed cross agglutination between the groups.

From table III it will be seen that 15 per cent. of the strains fell into the group with the O antigen VI. In this group neither urinary

TABLE III

Multiplicity of antigens on body and flagella of 75 (83·3 per cent. of strains) agglutinable paracolons when compared with their type strain

Agglutination		Number of strains								
		Agglutin-ated	Not agglutinated	Total	Agglutinated with somatic antigens		Total	Agglutinated with flagellar antigens		Total
Somatic	Flagellar				Single	Multiple		Single	Multiple	
+	+	3	...	...	3	...	...	3	...	...
+	−	23	...	...	23	...	...	...	...	...
+	++	22	...	...	22	...	...	...	22	...
−	+	8	...	...	...	...	...	8	...	...
−	++	8	...	...	...	...	...	...	8	...
++	++	6	...	...	...	6	...	...	6	...
++	−	5	...	...	...	5	...	...	...	...
−	−	...	15	...	...	...	...	...	...	...
Total		75	15	90	48	11	59	11	36	47
Percentage		83·3	16·7	100	81·4	18·6	100	23·4	76·6	100

− No agglutination.   + Agglutinated by one test serum only.
++ Agglutinated by more than one test serum.

nor fæcal strains predominated, nor were the members of this group isolated in any one epidemic. The other groups contained from 2·2 to 10 per cent. of the strains.

Biochemical and serological association

In table IV the distribution of the somatic antigens I–IX is compared with the biochemical classification into groups *a–p* (table I).

TABLE IV

Non-association of serological and biochemical grouping of 59 paracolon strains

Type strain	O antigen	Biochemical group	No of strains sharing O antigen	Biochemical groups of agglutinated strains										
				a	b	c	f	g	i	j	l	m	n	o
8144	I	a	5	1	2	2	...	...	...	...	'	...	...	...
1748	II	j	4	...	...	1	...	...	...	3	...	...	...	...
8179	III	c	3	...	...	3	...	...	...	...	...	...	...	...
1136	IV	i	4	1	...	1	...	...	2	...	...	...	...	...
4308	V, IX	i	9	1	1	1	...	...	4	2	...	...	...	...
1111	VI	i	22	...	...	...	1	1	13	2	1	...	1	3
7893	VII	m	6	1	...	3	...	...	...	...	...	1	1	...
7924	VIII	c	2	...	...	...	...	...	...	...	2	...	...	...
7973	IX	a	4	...	1	1	...	...	...	1	...	1	...	...

There is no constant association between the biochemical and serological groups; thus, only 27 (45·8 per cent.) of the strains belonging to the

biochemical group of the type strain shared the somatic antigen of that strain (shown in black type in table IV).

Flagellar antigen

Two recently isolated urinary strains and the *Bact. columbense* (N.C.T.C.) were tested in Tulloch (1939) tubes for diphasic variation. Forty discrete colonies from each strain were agglutinated to titre, which proved that these cells were monophasic.

Immunological response in man

The immunological response in man was tested in seven cases of cystitis. The freshly isolated strain was tested against the patient's serum. In two cases the titre was 1 in 400, in two it was 1 in 40, and in three it was less than 1 in 20. Eight faecal strains tested against the sera of the patients from whom they were isolated and against 20 sera sent for Wassermann reaction were not agglutinated to significant titres. It appears that man fails to develop antibodies to the paracolon bacilli which he harbours in the intestine. The sera of normal rabbits, on the other hand (Emslie-Smith, 1948 ; Schwabacher, 1949), contain paracolon agglutinins, but it is not known whether the antibodies result from the presence of the paracolon organisms in the gut.

A number of interesting anomalies may now be briefly noted.

Evidence for a heat-labile antigen in paracolon bacilli

The results of the mirror-absorption test with suspensions of 1136 and 8179 steamed at 100° C. are shown in table V. The unexpected feature of this test is the inagglutinability of steamed 1136

TABLE V

Mirror-absorption test of a pair of paracolon strains. (Absorbing and test suspensions steamed for 2 hours at 100° C.)

Serum (diluted 1 in 4 for absorption)	Absorbing suspension	Serum titre against test suspensions	
		1136	8179
1136	Nil	1280	<10
	1136	<10	<10
	8179	640	<10
8179	Nil	<10	640
	1136	<10	320
	8179	80	<10

suspension by unabsorbed 8179 serum and its agglutinability by 8179 serum after absorption with homologous 8179 suspension.

The most likely explanation appeared to be that 8179 serum, prepared against bacteria heated to 60° C., contained an antibody to a heat-labile antigen and that this antigen had not been completely

destroyed during the two hours' steaming of the test suspension of 1136. To test this point absorbed sera were titrated against suspensions treated in various ways. The suspensions were either living cells, cells that had been heated at 60° C. for one hour or cells that had been steamed at 100° C. for two hours. The corresponding titres were in the region of 640, 80, less than 20. Similar titres were obtained when these suspensions were tested against sera 1136 and 8179 after absorption with homologous suspensions treated by N HCl, thus giving positive evidence of a heat-labile antigen. Felix and Pitt (1936) and Kauffmann (1936) showed that serum absorbed by acid-treated bacilli will contain antibody to a heat-labile antigen and be free of antibodies to heat-stable O antigen. The agglutination of living bacteria was of the O type.

It is clear that the residual antibody in 8179 cannot be flagellar in type since the organism was non-motile and repeated attempts to demonstrate flagella by staining methods were unsuccessful. When serum 8179 was absorbed with acid-treated bacterium 8179, antibody was left for living 1136, a motile strain, but no agglutinin was found to formolised suspension of 1136. It was therefore concluded that the residual antibody in these two sera was the result of a heat-labile surface antigen on strains 1136 and 8179. Evidence was obtained that strain 1136 contained a greater quantity of this antigen than strain 8179.

As table V shows, steamed 8179 suspension was not agglutinated by the antibody to a heat-labile component, though serum 8179 clearly contained some. The difference between steamed suspensions 8179 and 1136 is possibly due to difference in amount or in heat lability, or both, of the heat-labile antigens. Whereas the M.A.D. of steamed 8179 for the removal of homologous agglutinins was 7×10^9 per ml., leaving agglutinins for the labile antigen of 1136, massive absorption of 8179 serum by a total of 160×10^9 per ml. removed all the 1136 agglutinins. If unheated 8179 or a suspension heated to 60° C. was used to absorb 8179, 14×10^9 per ml. was sufficient to remove antibody for 1136. On the other hand, the antibody in 8179 serum was removed by 40×10^9 per ml. of steamed 1136 as compared with 160×10^9 per ml. required of steamed 8179, showing that the labile antigen in steamed 1136 is present in larger amount than in steamed 8179.

The anomaly originally observed, namely that serum 8179 absorbed by a steamed suspension of its homologous strain 8179 agglutinated a steamed suspension of a heterologous strain 1136, is explicable on the supposition that the absorbed serum contains an unabsorbed antibody to a heat-labile antigen. That immune serum prepared with strain 8179 steamed at 100° C. for two hours failed to stimulate heat-labile antibody may be inferred from the fact that when the serum was absorbed by the steamed homologous suspension there was no residual agglutinin for living 1136.

Kauffmann (1936) recorded similar findings with *Salm. paratyphi-B* and *Salm. typhimurium*. Sera prepared with living organisms or with suspensions kept at 60° C. for one hour contained the antibody which remained after absorption of the sera with homologous suspensions steamed at 100° C. for two hours.

Felix and Pitt (1934) and Kauffmann (1935-36) showed that strains of bacteria containing Vi antigen which had been in the steamer at 100° C. for two hours had lost their Vi agglutinogenic properties, but that their power of absorbing Vi antibody had not been destroyed. A description of the absorption of antibody from sera 8179 and 1136 by massive doses of steam-killed suspensions has been given. By intraperitoneal inoculation, it was found that heat-labile antigen on strains 8179 and 1136 did not confer mouse pathogenicity.

DISCUSSION

Kauffmann (1941) reported on 4 strains of paracolons which shared the salmonella non-specific H antigens 1 and 5, and also the Vi antigen of *Salmonella typhi*. Three of these strains yielded opaque and translucent variants, the " V " and " W " forms of Kauffmann (1935-36). The opaque V colonies, agglutinated by Vi antiserum, were inagglutinable by O antiserum, and produced W variants. The translucent W colonies were inagglutinable by Vi antiserum, agglutinable by O antiserum, produced very few V variants, but absorbed Vi antibody. The fourth paracolon isolated with Vi antigen grew as a W variant.

Kauffmann (1943, 1944a, b, c, d) has recorded a new heat-labile somatic antigen, " L " antigen, on *Bact. coli* and paracolon bacilli. This L antigen resembles the Vi antigen in that it is destroyed at 60° C., but differs in that suspensions killed at 60 or 100°, or by alcohol, fail to absorb homologous agglutinin from antisera. To prepare an antiserum to the L antigen it is necessary to choose a culture which when tested in living suspension against the O antiserum fails to be agglutinated, but when tested with suspension killed at 100° C. is agglutinated. This strain used in the viable state to immunise a rabbit will yield an anti-OL serum. In testing for L antigen-antibody union, living bacteria must be used and the agglutinable suspension incubated for 20 hours at 37° C.

L antigen cultures contain " L plus " opaque variants and " L minus " translucent variants which are entirely comparable to the V and W variants of Vi cultures. Using mice, Kauffmann showed that inocula of from 50×10^6 to 500×10^6 L plus organisms were lethal, whereas L minus cultures in similar dosage failed to kill a high proportion of mice. By active and passive immunisation of mice, he was able to ascribe protective properties to the L antigen.

It follows that by testing coliforms against L plus and L minus sera a technique has been found by which virulent strains of coliforms

can be detected. Knipschild (1945, cited by Vahlne, 1945) in collaboration with Kauffmann, demonstrated that the L antigen is a capsular antigen which Vahlne (1945) renamed the K antigen. Immune K serum produces capsular swelling, similar to that produced by pneumococcal antiserum, when in contact with homologous *Bact. coli*, and 1515 strains of *Bact. coli* and *Bacterium aerogenes* were successfully grouped into 115 types on the basis of their O, H and K antigenic analysis. Analogous methods applied to paracolons might produce similar results.

Marmion (1944) reported 2 paracolon strains which were agglutinated to titre by *Salm. typhi* Vi serum. Five other reported strains are probably descendants of Kauffmann's strains, so that a total of 6 and perhaps 11 strains has been recorded as containing a heat-labile antigen. To this total, strains 1136 and 8179 may now be added as having an antigen comparable in heat-lability with the Vi antigen.

Combining but non-agglutinating antibodies in paracolon antisera

In the mirror-absorption test of strains 8179 and 1136 (table V) there is evidence of a masked antigen-antibody reaction which needs explanation. The unabsorbed serum 8179 failed to agglutinate heterologous bacteria with the heat-labile antigen on their surface until the serum had been absorbed by the homologous strain. We may suppose that the suspension of 8179 absorbed a non-agglutinating antibody, leaving an agglutinating antibody to react with the corresponding heat-labile antigen in 1136.

This phenomenon recalls Ehrlich's hypothesis of agglutinoids which Shibley (1929) showed could be produced artificially by heating the serum. This model postulates that agglutinoids are more avid for organisms than agglutinins. Leaving aside this question of avidity, the phenomenon might be due to the agglutinogen being rendered insusceptible in some way to the agglutinin. For example, the protection of fœtal erythrocytes from maternal α and β agglutinins is believed to be affected by water-soluble group-A and -B substances present in the infant's tissue uniting with the maternal agglutinins that reach the fœtus. The observation of Bawden and Kleczkowski (1941, 1942) is an example of antibody failing to show macroscopic evidence of antigen-antibody union. Heating serum-albumin and serum-globulin produced an albumin-globulin complex that proved to be antigenic. The antiserum to the complex precipitated with globulin alone ; the precipitate was inhibited by the complex.

The anti-paracolon sera were not knowingly subjected to processes likely to produce such conjugation, but as Wilson and Miles (1946) visualise the possible occurrence of antigenic but non-reacting substances in nature, the anomalous reaction of the paracolon serum is worth recording, despite the absence of an explanation.

SUMMARY

1. Ninety paracolon bacilli were divided into 16 biochemical groups by their power to ferment sucrose, dulcitol, salicin and lactose. Immune serum was prepared from 9 strains representing the predominant biochemical group and the antigenic relationships of these strains were determined by mirror-absorption tests.

2. Of 90 late-lactose fermenters from fæcal and urinary sources, 75 (83·3 per cent.) shared either a somatic or a flagellar antigen, or both, with one or more of the type strains. It was possible to characterise 59 (65·5 per cent.) strains in terms of a somatic antigen. The somatic antigen of 18·6 per cent. of these 59 strains was shared by more than one of the type strains. Forty-seven (52·2 per cent.) had H antigens related to those of the type strains. There was multiple cross agglutination in 76·6 per cent. of these 47 strains, showing the lesser specificity of flagellar antigens.

3. There was no constant association between biochemical and serological groups, nor was there an antigen common to all or even the majority of the paracolon strains.

4. Three strains of motile paracolon bacilli tested for phase variation were apparently monophasic.

5. Fifteen strains of non-lactose fermenters were tested against the serum of the patients from whom the organisms had been obtained. Two agglutinating systems yielded a significant titre.

6. Two strains of paracolon bacilli, 1136 and 8179, were found to possess a common heat-labile antigen similar to the non-virulent Vi antigen of Salmonella described by Kauffmann (1936). The proportional amount of the Vi antigen appears to vary with the strain. It was present in greater quantity on 1136 than on 8179.

7. One rabbit antiserum appeared to contain non-agglutinating antibodies to a strain containing an agglutinable heat-labile antigen.

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THE PATHOLOGY OF SUBACUTE COR PULMONALE IN DIFFUSE CARCINOMATOSIS OF THE LUNGS

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(Plates XIX-XXIII)

The main object of this paper is to describe a case of lymphangitis carcinomatosa of the lungs complicating an undiagnosed gastric carcinoma and giving rise to obliterative endarteritis of the pulmonary vessels, with right ventricular hypertrophy. The literature of lymphangitis carcinomatosa and subacute cor pulmonale is briefly reviewed, and in the discussion the inter-relation of the various pathological processes is considered.

Case Report

Clinical history

The patient, a male aged 58, a cafe proprietor, was admitted to Westminster Hospital with cyanosis, dyspnœa and œdema and died 4 days later.

Past history. Never robust, he had been subject to bronchitis for many years. At the age of 31 a gastro-enterostomy was performed for duodenal ulcer. Over an indeterminate period he had been breathless on exertion, especially in winter, when he suffered from colds, and 5 years before his death, chronic emphysema was diagnosed radiologically. At this time he began to complain of a dull aching pain in the epigastrium, radiating round the right hypochondrium, and for the last 3 years of his life he continued to have pain and flatulence, apparently unrelated to food. During the last year existence was a misery because of daily attacks of abdominal pain, though he never vomited. X-ray examination of the chest 18 months before death revealed gross emphysema, with cor pulmonale (fig. 1) : a barium meal showed a deformed duodenal cap and pylorospasm, suggestive of continued duodenal ulceration.

Recent history. During the last few months of his life he continued to attend as an out-patient on account of abdominal pain and loss of weight. A barium meal 7 weeks before death showed the stoma functioning well after some initial delay, but the presence of a tumour was not suspected ; a fractional test-meal, however, showed achlorhydria, even after histamine. One month before admission, following a " cold," breathlessness became greatly increased : two weeks later he complained of intermittent pain in the chest. A few days before admission he developed orthopnœa and dependent œdema.

On examination he was wasted, orthopnœic and cyanosed, with dilated venules over the nose and cheeks, but the neck veins were not distended. He

had slight œdema round the eyes, and œdema of the elbows and hands, sacrum and ankles. Pulse weak, 96/minute : B.P. 110/75. Auscultation revealed a triple rhythm, heard most clearly in the epigastrium. The chest was emphysematous, with less than half an inch of expansion, hyper-resonance, and diminution of cardiac and hepatic dulness. Air entry was poor ; rhonchi were audible in all areas and râles at both bases. The liver, which appeared to be tender, was palpable 4 in. below the costal margin. X-ray examination of the chest showed fine mottling of the upper and middle zones (fig. 2).

Before death, the œdema disappeared from the face and arms and settled in the lower limbs, but the cyanosis and dyspnœa increased. He died 4 days after admission.

Post-mortem examination

(22½ hours after death)

A spare elderly male with œdema of both ankles. The chest was not barrel-shaped.

Stomach. A totally unexpected finding was a sclerosing carcinoma of the leather-bottle type, involving the distal half of the stomach, extending proximally along the lesser curvature and distally as far as the pyloric sphincter. In the centre of the area of growth the old gastro-enterostomy stoma was widely patent, the tumour apparently involving the gastric but not the jejunal side of the opening. Along the lesser curvature was a crop of small white lymph-nodes invaded by growth. *Liver, spleen* and *kidneys* showed well-marked passive congestion but no signs of metastases.

The *heart* weighed 370 g., the increase being due entirely to hypertrophy of the right ventricle, which showed thickening of the columnæ carneæ and papillary muscles (fig. 3). The thickness of the right ventricular muscle, midway between base and apex, was 1·0 to 1·3 cm. The left ventricle at the same level varied from 1·1-1·5 cm. in thickness. The tricuspid and pulmonary valve-rings were dilated, but the cusps themselves were healthy. Mitral and aortic valves normal. The aorta showed slight atheroma.

Both *lungs* were bound to the chest wall by old adhesions ; their anterior margins were ballooned at two or three points into thin-walled bullæ, but otherwise were not strikingly emphysematous, and certainly not severe enough to be associated with cor pulmonale. When palpated, they did not crepitate ; on the contrary their consistence was rather tough.

The cut surface, when examined with a hand lens, showed patches of emphysema and a general increase in stroma, with short white fibrous cords, seen on cross section as white dots, running through the lung substance (fig. 4). There was no other macroscopic evidence of carcinomatous deposits, and the pleura appeared normal. The thoracic duct and hilar lymph-nodes were macroscopically normal. The pulmonary arterial tree was dilated throughout, and the larger branches showed broad plaques of atheroma.

COR PULMONALE IN CARCINOMATOSIS OF LUNGS
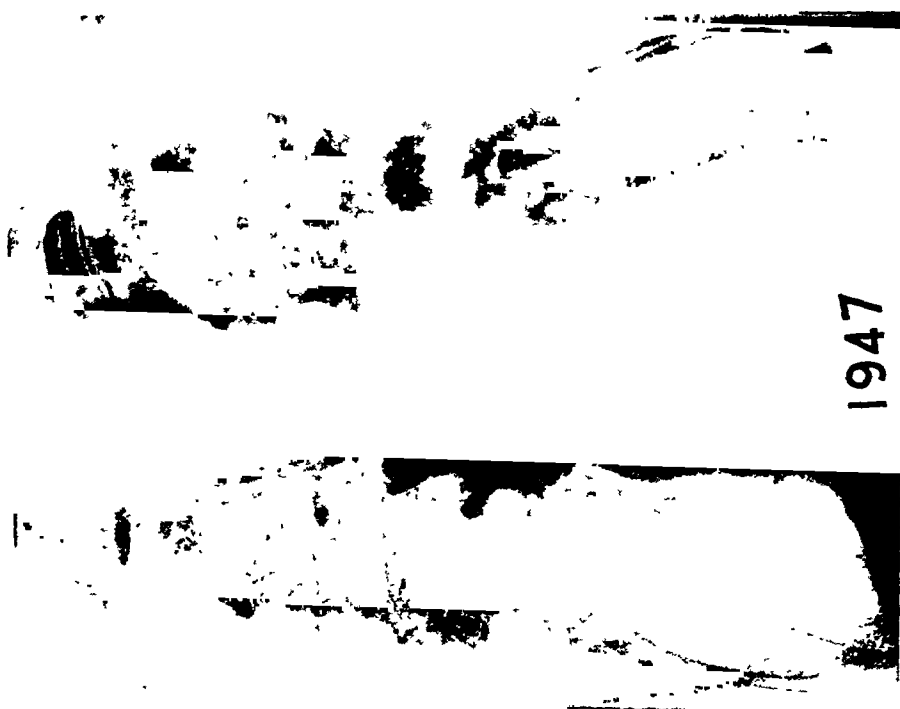
FIG. 2.— X-ray of chest (portable) showing mottling of upper two-thirds of lungs (3 days before death).
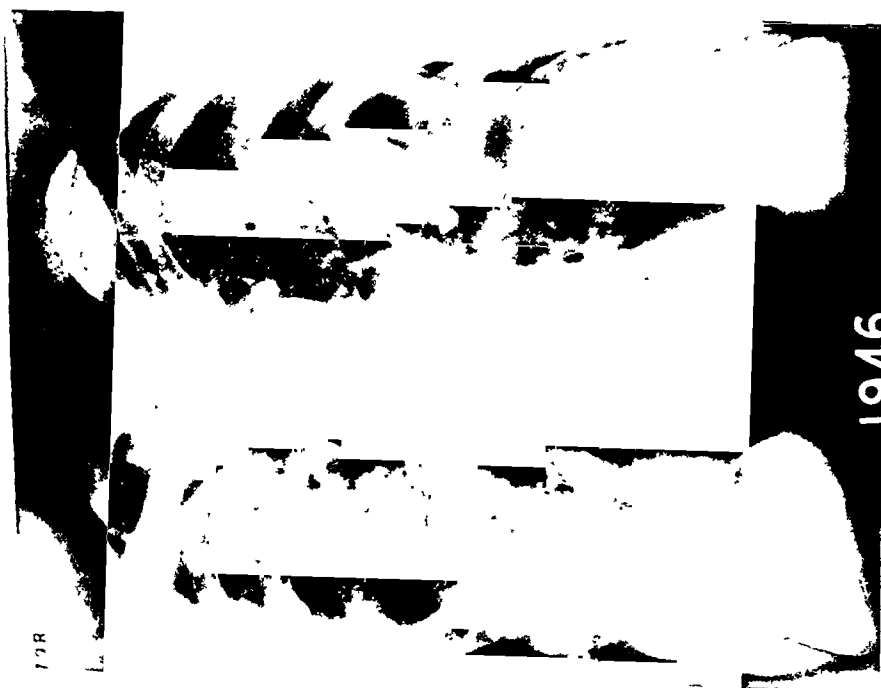
FIG. 1.—X-ray of chest showing emphysema and cor pulmonale (18 months before death).

Histology

Stomach. The tumour had the usual characters of an atrophic scirrhous carcinoma, the great bulk of the growth being composed of fibrous tissue throughout which small, darkly-staining anaplastic cancer cells were sparsely scattered.

Lungs. The microscopic picture was as extraordinary as it was unexpected. Two main changes were observed :—(1) The great majority of the arterioles were completely occluded by a form of obliterative endarteritis. (2) The perivascular and peribronchial lymphatics throughout both lungs were distended by plugs of cancer cells (lymphangitis carcinomatosa).

Nearly all the arteries less than 0·4 mm. in diameter were completely occluded, mostly by well-formed connective tissue, a few by more or less recent thrombosis. Vessels between 0·4 and 0·8 mm. were partially occluded by fibrous intimal processes apparently growing from the wall (fig. 5), while the larger vessels, though they showed perivascular carcinosis, had a clear lumen. The intervening alveoli were free of tumour cells, and showed foci of emphysematous dilatation, presumably " compensatory " (figs. 6 and 7).

The appearance of the small arteries, on cross section was variable. The majority were converted into solid cords (forming the network seen macroscopically) due to extensive and long-standing intravascular and perivascular fibrosis. These cords were permeated by cancer cells, in some cases intravascular and perivascular (fig. 8), in others perivascular but not intravascular (fig. 9), and in others intravascular but not perivascular, the picture obviously varying with the plane of section. It was not possible, however, to demonstrate a connection between the cancer cells in the lumen and those in the perivascular lymphatics, the media being always intact. Even in the thin-walled pre-capillary vessels this was so (fig. 10). Evidence of the long-standing nature of the lesion was afforded by the degree of atrophy and absorption of the media at certain points. In a few instances it had completely disappeared, its site being indicated by a ring of carbon-pigment in the thickened adventitia.

Giant sections showed that these changes were more marked in the peripheral and subpleural zones of the lung than in the hilar region, although some of the hilar lymph-nodes showed early invasion. Weigert's elastic stain showed increase in the elastic laminæ of the larger arteries (fig. 11) due to pulmonary hypertension.

LYMPHANGITIS CARCINOMATOSA

In 1868 Bristowe described the post-mortem appearances in a man dying from cancer of the lower œsophagus and cardia, in which the subpleural lymphatics were distended with cancer cells, forming a fine network on the lung surface. Microscopically the lungs showed widespread dissemination of tumour cells in the lymphatic system,

and he suggested that retrograde spread of the tumour had occurred throughout the pulmonary lymphatics from metastases in the hilar glands.   Three years later Bennett (1871) recorded similar appearances in the lungs of a patient 2 years after removal of the left breast for scirrhous cancer, and added that the cut surface of the lungs resembled that seen in miliary tuberculosis, apparently due to fibro-cancerous growth around the blood-vessels and bronchioles.   Troisier (1873) and Girode (1889) gave accounts essentially similar, the latter using the term "cancerous lymphangitis," and recording traces of "endarteritis" in the pulmonary arterioles, which he appears to have regarded as a coincidental finding.   He suggested three possible modes of spread to the lungs :—(1) *Hæmatogenous*, following invasion of the veins in the vicinity of the tumour ;   cancer cells were thus conveyed via the right heart to the pulmonary arterioles, with subsequent invasion of the surrounding lymphatics.   (2) *Direct*, through the diaphragm, followed by invasion of the pulmonary lymphatics through the pleural stomata.   (3) *Lymphogenous*, to the thoracic duct and hilar lymph-nodes, with retrograde invasion of the pulmonary and pleural lymphatics.

Schmidt (1903) published a series of cases in which one-half revealed the presence not merely of endarteritis but of cancerous emboli in the pulmonary arterioles, a finding which led him to conclude that access to the lungs was obtained through the thoracic duct, great veins, right heart and pulmonary arteries.   He pointed out that the absence of invasion of the thoracic duct did not invalidate his theory, since a patent duct was more likely to transmit emboli than one occluded by tumour.   In one of his cases hypertrophy of the right ventricle was observed, and in 1919 von Meyenburg described two similar cases with right ventricular hypertrophy, suggesting as a cause obstruction of the pulmonary arterioles, either by obliterative endarteritis, or by compression of the vessels by tumour masses in the perivascular lymphatics.   He regarded the presence of cancer cells in the vascular lumen as a secondary phenomenon, due to invasion of the vessel wall from without.   Schierge (1922) considered it possible for blood-borne tumour cells to pass through the pulmonary capillaries into the lymphatics without being arrested, and it is interesting to note that most accounts of lymphangitis carcinomatosa do not record the presence of tumour cells within the blood-vessels themselves.

Obliterative endarteritis

Schmidt in his series appears to have regarded the fibrous occlusion of the pulmonary arterioles as an end-result of organisation of thrombi precipitated by cancerous embolism.   von Meyenburg, on the other hand, described an obliterative endarteritis which he considered a reaction on the part of the intima to the presence of tumour cells in the perivascular lymphatics.   This is the view of Greenspan (1934)

and Brill and Robertson (1937). In an admirable review of 49 cases of lymphangitis carcinomatosa, including most of the foregoing, Wu (1936) pointed out that only 8 showed endarteritis, and that 5 of these were associated with cancerous emboli in the pulmonary arterioles. Therefore, though he regarded lymphangitis carcinomatosa as a retrograde lymphatic spread from the hilum, he considered that intravascular deposition of cancer played an important part in the genesis of the obliterative changes.

Respiratory symptoms in lymphangitis carcinomatosa

The main clinical features are increasing dyspnœa, frequently proceeding to orthopnœa, cough, expectoration and sometimes chest pains. Physical signs are disproportionately few, the chest being resonant to percussion, and in several cases emphysematous (Rössle, 1908 ; Bernard and Cain, 1913 ; Durbeck, 1926 ; Mason, 1940). Dry pleurisy and pleural effusion have been recorded. Cyanosis is usually severe. The average duration of respiratory symptoms is from 3 to 8 weeks. It is generally agreed that they are cardiac in origin, and not due, as was thought by Girode (1889) and Bard (1906), to œdema of the lungs from lymphatic obstruction.

SUBACUTE COR PULMONALE

In Wu's series, 11 of the 49 showed right ventricular hypertrophy. In 1937 Brill and Robertson introduced the term subacute cor pulmonale to indicate a right-sided hypertrophy occurring in association with diffuse carcinomatosis of the lungs, developing during the last few weeks of life, and not attributable to any other cause. The consensus of opinion favours arteriolar blockage as the cause of right ventricular hypertrophy, although it is considered that compression of the vessels by tumour cells in the perivascular lymphatics may play a part. Thompson and White (1936) estimate that at least 2 months' strain is necessary to produce appreciable hypertrophy of the right ventricle.

Genesis of subacute cor pulmonale : analysis of cases

Study of an adequate number of cases makes it clear that there is a type of diffuse carcinomatosis of the lungs, complicating cancer arising in the abdomen, in which the abdominal symptoms are either absent or largely overshadowed by respiratory distress. At autopsy the lungs at first sight appear to be free from involvement, but closer inspection may reveal one or both of the following :—(1) A whitish network of distended lymphatics on the pleural surface (fig. 12). (2) Numerous fine white cords forming a tracery on the cut surface of the lung, appearing on cross section as small white dots. These

give rise to X-ray appearances sufficiently characteristic to enable a diagnosis to be made, sometimes months before the primary growth has declared itself (Lorenz, 1921-22 ; Lucas and Pollack, 1931).

During the last century nearly 100 cases have been recorded, but inevitably the absence of morbid anatomical or histological data renders a number of these unsuitable for consideration. Fairly comprehensive lists have been compiled in papers by Poppi (1935) and Wu (1936). With a view to correlating the various pathological changes underlying the clinical symptoms, 78 of these have been analysed, with the following results.

Site of primary. In about three-quarters of the cases the stomach was the seat of a primary carcinoma, seldom diagnosed during life. Other sites included the breast, prostate, bronchus, biliary apparatus, pancreas and pelvic organs.

Distribution of tumour cells in lungs. Microscopically 69 showed tumour cells in the pulmonary lymphatics (lymphangitis carcinomatosa). The detail given in the histological reports is variable, but no less than 22 of the 69 recorded the presence of cancer cells inside the blood-vessels also, while only 5 authors stress the absence of this phenomenon. Nine cases showed tumour cells in the vessels only, without evidence of lymphangitis carcinomatosa. There is thus no clear-cut histological distinction between the group which is clearly blood-spread, and that which is held to be lymph-spread.

Pulmonary arteriolar occlusion. From the descriptions and photomicrographs it is apparent that what is " endarteritis " to one author is merely fibrous organisation of thrombi to another. I shall therefore use the term " intravascular fibrosis " to cover both senses. What is more important is that in 40 cases there is mention of an obstructive lesion in the pulmonary arterioles, 30 in the form of intravascular fibrosis and 10 from cancerous embolism with or without recent thrombosis.

Right ventricular hypertrophy was found in 11 cases, in only one of which was the diagnosis made during life. The relevant details are summarised in the accompanying table.

Since only a handful of cases of lymphangitis carcinomatosa showed cor pulmonale, it is not likely that the presence of tumour cells in the perivascular lymphatics alone will produce right ventricular hypertrophy, as has been suggested by Goldmann (1931). A glance at the table, on the other hand, shows a significantly high incidence of intravascular fibrosis (8) or more recent occlusion by tumour cells with or without thrombosis (2).

Intravascular fibrosis. Twenty-four of the 30 cases showing intravascular fibrosis also showed tumour cells in the lumen of the vessel, suggesting that it is the cancer cells which give rise to the thrombosis. The high incidence of scirrhous tumours lends weight to the suggestion of Brill and Robertson that the type of the growth itself may be a factor in determining the degree of intravascular fibrosis. This is

PLATE XX

COR PULMONALE IN CARCINOMATOSIS OF LUNGS

FIG. 3.—Transverse section of heart showing hypertrophy of right ventricular muscle.
×1·3.
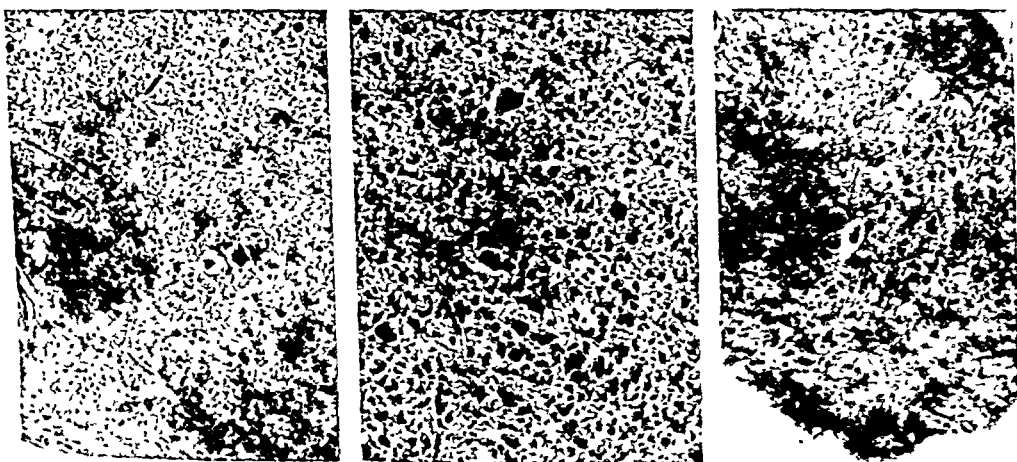
FIG. 4.— Magnified view of texture of lung (right) compared with normal lung (left) and chronic emphysema unassociated with cor pulmonale (centre). ×2.

PLATE XXI

COR PULMONALE IN CARCINOMATOSIS OF LUNGS
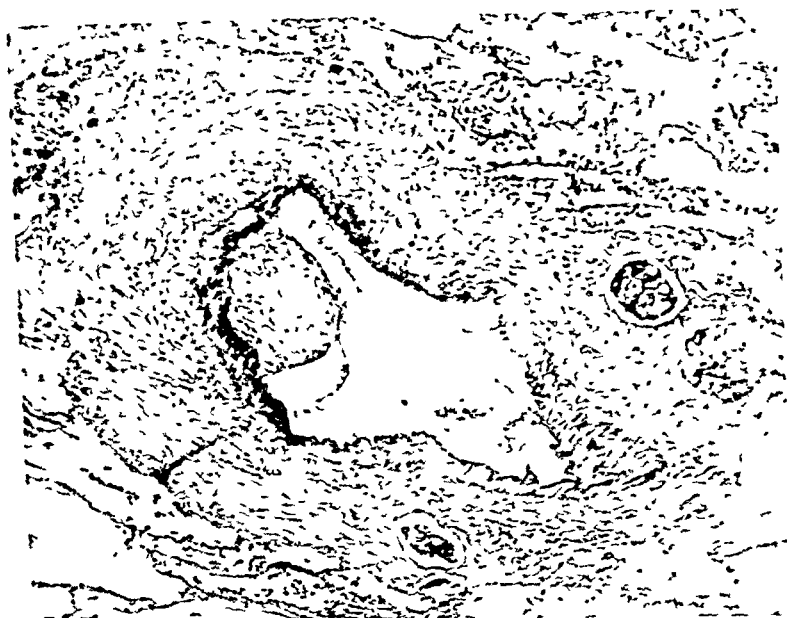
FIG 5 —Pulmonary vessel showing fibrous process growing from intima, with perivascular fibrosis and carcinosis   Hæmatoxylin and eosin   ×75
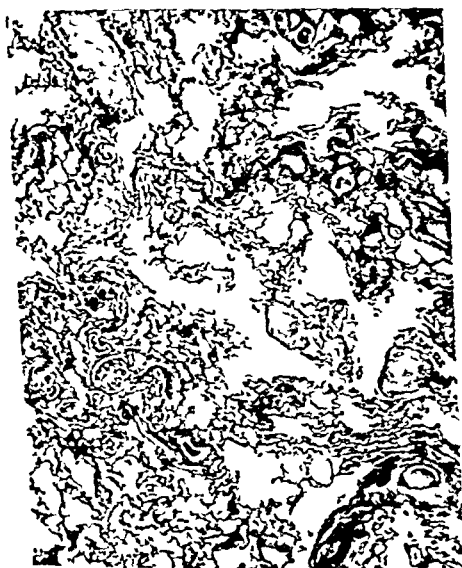
FIG 6 —Low power view of lung showing obliteration of small vessels and focal emphysema   Hæmatoxylin and eosin   ×9

FIG 7 —Low power view of lung showing groups of cancer cells in perivascular lymphatics   Hæmatoxylin and eosin   ×9

COR PULMONALE IN CARCINOMATOSIS OF LUNGS

FIG. 8.—Pulmonary vessel occluded by intravascular fibrosis, and showing both intravascular and perivascular carcinosis. Hæmatoxylin and eosin. × 100.
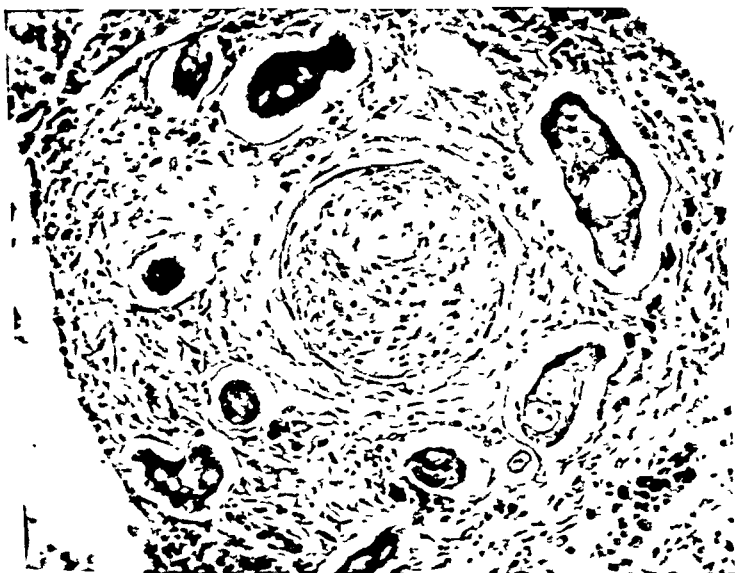
FIG. 9.—Occluded arteriole showing perivascular but not intravascular carcinosis. Hæmatoxylin and eosin. × 125.

COR PULMONALE IN CARCINOMATOSIS OF LUNGS

FIG. 10.—Pre-capillary vessel containing a cancerous embolus, with tumour cells in the perivascular lymphatics. Hæmatoxylin and eosin. ×125.
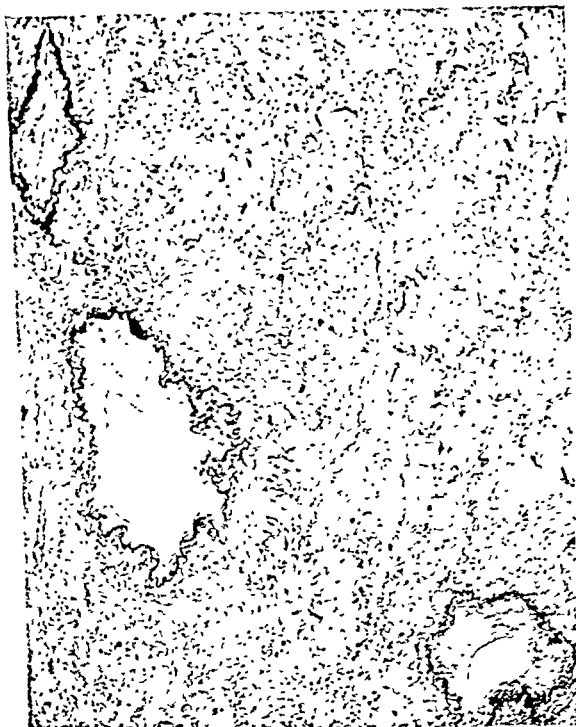
FIG. 11.—Lung showing pulmonary arteriosclerosis. Weigert's elastic stain. ×75.
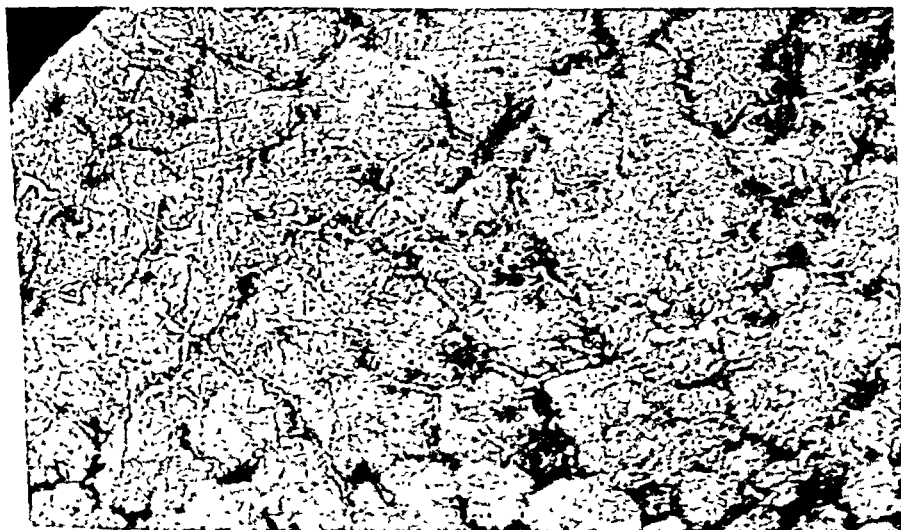
FIG. 12.—Magnified view of lung surface showing a network of pleural lymphatics distended by cancerous permeation (lymphangitis carcinomatosa). ×2.

certainly true of the perivascular fibrosis, since there are no other factors to account for it.

TABLE

Diffuse carcinomatosis of the lungs: relation of right ventricular hypertrophy to other changes

Case no	Site of primary tumour	Type of tumour	Lymphangitis carcinomatosa	Intravascular fibrosis	Recent thrombosis	Cancer cells inside vessels
1	Stomach	Scirrhous	—	±	...	—
2	,,	,,	±	+	÷	÷
3	,,	Encephaloid	+	+	÷	+
4	,,	Scirrhous	±	—	÷	+
5	,,	Polypoid	⊤	...	...	...
6	,,	Scirrhous	±	±	±	±
7	,,	,,	±	+	±	...
8	,,	,,	—	+	÷	±
9	,,	Anaplastic	±	—	÷	—
10	Breast	Spheroidal celled	—	÷	÷	+
11	Unknown	..	÷	+	...	...

THE SPREAD OF CANCER IN THE LUNGS

Opinion varies as to how the tumour cells reach the lungs. The theory of retrograde lymphatic spread in every case is open to several objections. (1) It conflicts with the findings of Costedoat and Codvelle (1932) who, in an excellent review of the clinical and pathological picture of lymphangitis carcinomatosa, pointed out that the thoracic duct at autopsy was usually clear. (2) It does not satisfactorily account for the presence of tumour cells inside the blood-vessels in 30 per cent. of cases. (3) Nine cases in the above series (including 2 with right ventricular hypertrophy) were certainly blood-spread and failed to show tumour cells in the lymphatics at all.

If one postulates hæmatogenous spread, two questions present themselves. How do the tumour cells reach the bloodstream and how do they reach the pulmonary lymphatics ? In the absence of hepatic metastases it is likely that invasion of the bloodstream is not via the gastric veins, but by way of the thoracic duct and systemic veins (Stevens, 1907) and so through the right heart into the pulmonary artery.

Several authors are of the opinion that spread to the perivascular lymphatics of the lungs occurs by direct extension through the arteriolar wall, and there is no doubt that in the more nodular forms of pulmonary carcinosis this sometimes occurs. In the case described above, however, the media was invariably intact, though the appearances suggest that if one could follow the vessels to their finest ramifications, an unbroken communication between the cancerous emboli in the vessels and the tumour cells in the lymphatics might eventually be traced. Indeed, such continuity was traced in one of Schmidt's series.

It would be unwise to draw a general inference from a single instance, but in the present case the variable appearances would bear explanation on the basis of the following mode of spread :— Small tumour emboli in the pulmonary circulation become arrested in the smaller arterioles, and thereafter the tumour cells spread along the vessel, both proximally and distally, causing thrombosis and intravascular fibrosis. On their reaching the smallest vessels ready access to the lymphatic system of the lungs is obtained, thus accounting for the subpleural, perivascular and peribronchial network. That is to say, the lymphatic spread is towards the hilum, and the hilar glands are involved later. Large-size sections in the present case show the infiltration to be more marked in the periphery of the lung than the hilum, which tends to support this view.

A random section from a recent case of lymphangitis carcinomatosa (due to primary prostatic cancer) clearly shows tumour cells in the subpleural lymphatics and lymph-spaces in direct continuity with the perivascular lymphatics of a small vessel. This might equally be taken to indicate retrograde lymphatic spread, but again the periphery of the lung was more heavily involved than the hilar region.

The present case would appear to be exceptional in certain respects, notably the duration of symptoms. Five years before death the patient was radiologically emphysematous, and cor pulmonale was noted 18 months before the end, *i.e.* it was not truly " subacute." Now emphysema, even though advanced, does not ordinarily produce cor pulmonale (White and Brenner, 1933) ; moreover the lungs at autopsy were not those of chronic vesicular emphysema, the patchy alveolar dilatation being presumably an attempt to compensate for the reduced vascular bed. As stated, clinical emphysema has been noted in several cases of lymphangitis carcinomatosa but was usually of short duration.

In view of the duration and degree of the right ventricular hyper-trophy and the extent and chronicity of the pulmonary arteriolar changes, one is obliged to consider the possibility that the insidious nature and slow growth of the primary tumour determined the rate of subsequent events. Linitis plastica is extremely slow in its growth —Costedoat and Codvelle record a case dying 8 years after gastrectomy —and its metastases may be equally slowly growing. The arteriolar changes in the lungs, notably the complete disappearance in places of the musculo-elastic media, must have taken place over a fairly lengthy period, and one must therefore entertain the possibility that the pulmonary circulation was subjected to a sparse but steady shower of tumour emboli over a period, not of weeks or months, but of years.

SUMMARY

1. A case of lymphangitis carcinomatosa with right ventricular hypertrophy is described, the primary tumour being a scirrhous carcinoma of the stomach.

2. A study of the literature of diffuse carcinomatosis of the lungs reveals that in three-quarters of the cases the primary tumour was a gastric carcinoma, seldom diagnosed during life.

3. Analysis of the microscopical reports in 78 published cases shows that there is no clear-cut histological distinction between the group where spread to the lungs is obviously *hæmatogenous* and that which is held to be due to retrograde spread from the hilar lymphnodes (lymphangitis carcinomatosa). One-third of the cases of lymphangitis carcinomatosa showed tumour cells in the blood-vessels as well as in the perivascular lymphatics, frequently associated with diffuse obliterative endarteritis or organised thrombosis.

4. Of the eleven cases accompanied by right ventricular hypertrophy, ten showed an obliterative lesion of the pulmonary arterioles in the form of intravascular fibrosis or more recent thrombosis. There is thus reason to believe that subacute cor pulmonale is due, not to lymphangitis carcinomatosa *per se*, but to occlusion of the pulmonary arterioles.

5. The suggestion is made, in the light of these findings, that lymphangitis carcinomatosa follows a hæmatogenous spread of tumour cells to the lungs rather than a retrograde spread from the hilar lymphatics.

I am indebted to Dr W. E. Lloyd for the clinical details; to our Department of Medical Photography for the photographs; and to Professor R. J. V. Pulvertaft for the photomicrographs.

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THE ROLE OF TEST TOXINS AND THE NEED FOR STANDARDS IN THE DETERMINATION OF AVIDITY OF ANTITOXINS

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IT was suggested by Glenny and Barr (1932) that the simplest measure of avidity for diphtheria antitoxin was the dilution ratio. This ratio is the amount of antitoxin required to neutralise the Lr dose of toxin in a total volume of 2 ml., divided by the amount necessary to neutralise this dose in a volume of 200 ml., as determined by the guinea-pig intracutaneous method of test. These levels of testing correspond respectively to Lr/10 and Lr/1000, and the sera for which the amount required to neutralise is greater at the more dilute level are classed as non-avid, because their combination with toxin is not firm and dissociation occurs at high dilutions. A correlation was shown to exist between the serum ratio (in-vivo value : in-vitro value), the results of combining-power tests by the rabbit intravenous method (Madsen and Schmidt, 1930) and the value of the dilution ratio against a certain fixed toxin.

Later work has shown that, as might be expected, the value of the dilution ratio for any given antitoxin varied according to the batch of toxin used for the titrations.

Experimental

This paper is concerned with the results of dilution-ratio determinations of antitoxins of different grades of avidity, using for the titrations 5 toxins of different degrees of toxicity as measured by the number of M.R.D. present in the Lr dose. Of the toxins, prepared by Dr Linggood, three were produced from routine P.W. 8 strains, the times of growth being 4, 10 and 10 days. One of these 10-day toxins, A, had been in the store for a number of years, but all other preparations were freshly made. A further two toxins were produced from *gravis* strains and grown for 3 and 10 days. Details of the 5 toxins are given in table I, which shows that the relative toxicity of the short-growth *gravis* preparation, E, was considerably greater than that of any of the other toxins, A and B being almost identical, and C and D more toxic than A and B but much less so than E. A

comparison of the data for B and C suggests that less natural toxoiding occurs during the growth of the *gravis* strain. The *gravis* toxins, being of low potency, were concentrated by ultra-filtration.

TABLE I

Some details of the diphtheria toxins used in these experiments

Toxin						A	B	C	D	E
Strain .					.	P.W. 8	P.W. 8	*gravis*	P.W. 8	*gravis*
Length of growth (days)					.	10	10	10	4	3
Toxicity $\frac{\text{(M.R.D. per Lr dose)}}{10^4}$					.	3·2	3·5	5·5	6·2	15·0
Age .		.	.	.	.	Old	Fresh	Fresh	Fresh	Fresh

The samples of antitoxin selected, 14 in all, covered a range of serum ratios from 0·77 to 4·29, and the dilution ratios of these were determined against each of the five toxins shown in table I. The resulting values are given in table II. The last column shows that

TABLE II

Values of the dilution ratio for 14 sera, each determined against 5 toxins

Serum	Serum ratio	Dilution ratio against toxin					Average dilution ratio
		A	B	C	D	E	
1	1·60	1·18	1·09	0·98	1·00	0·98	1·046
2	4·29	1·20	1·07	1·00	1·00	0·91	1·036
3	1·29	1·12	0·98	0·94	0·94	0·91	0·978
4	1·59	1·12	0·95	0·96	0·90	0·83	0·952
5	1·25	1·04	0·94	0·95	0·89	0·85	0·934
6	1·21	1·06	0·88	0·98	0·88	0·81	0·922
7	2·38	1·03	1·05	0·83	0·84	0·69	0·888
8	1·38	1·09	0·92	0·84	0·81	0·77	0·886
9	0·99	0·99	0·99	0·83	0·84	0·74	0·878
10	1·11	0·97	0·94	0·84	0·82	0·65	0·844
11	1·27	0·97	0·83	0·75	0·78	0·69	0·804
12	0·98	0·90	0·80	0·77	0·74	0·70	0·782
13	0·77	0·77	0·68	0·59	0·63	0·59	0·652
14	0·80	0·82	0·62	0·66	0·61	0·53	0·648
Average .		1·018	0·910	0·851	0·834	0·761	

the sera are arranged in descending order of dilution ratio, and the average figure for the 14 sera, calculated for each toxin and given on the last line in table II, shows that the dilution ratio of the sera appeared highest when toxin A was used, the average decreasing progressively from A to E. The difference between the two extremes A and E is approximately 25 per cent., and it is evident that the higher the toxicity of the test toxin the lower the dilution ratio of the antitoxin, a result which might be expected from theoretical considera-

tions. The greatest consecutive difference, however, is between A and B, although little difference in toxicity could be demonstrated : the difference between D and E is nearly as great, and that between B and C greater than that between C and D. Nearly all sera appeared avid or of average avidity against toxin A, while all appeared non-avid against E. Thus the trend of values for dilution ratio against the 5 toxins is indicated by the following :—

Toxin	No. of antisera with dilution-ratio values	
	<0·80	>0·90
A	1	12
B	2	9
C	4	6
D	4	4
E	8	3

It is evident from table II that no absolute value of the dilution ratio can be allocated to any serum and that the avidity of a serum should be referred to that of an " avidity standard ", the dilution ratio of which should be measured from time to time against the test toxins in current use. The dilution ratio of the standard against any given test toxin would constitute the " dilution characteristic " for that particular toxin.

Table III gives a summary of the relative avidity of sera 1-14 in table II referred to the general dilution characteristic of each toxin, *i.e.* the average of the dilution ratios of all 14 sera tested against each toxin. Dilution ratios referred to some standard figure will be termed " dilution indices." It is evident from table III that the majority of the calculated indices against 5 different toxins are within experimental error of the average for each serum. In practice it is necessary to select a serum to be used as a standard, and examination of the results in table III suggests that serum 2 or serum 12 would be the most suitable antitoxins for this purpose. Serum 2 is to some extent abnormal, having a serum ratio of 4·29 : for immediate purposes, therefore, serum 12 has been chosen as avidity standard, and table IV gives the average dilution ratio of the remaining sera, referred to the average value for serum 12 (column 2). The remaining columns give values of the percentage deviation from the average index for each toxin used, and the average percentage deviation for the five determinations.

It will be seen that 55·7 per cent. of the values are within 2·5 per cent. of the average, 78·6 per cent. within 5 per cent., 87·1 per cent. within 7·5 per cent., and 92·9 per cent. within 10 per cent. These figures show that when sufficient tests are made, usually five for each determination, the results are correct within very narrow limits, but the distribution of the values suggests that there are some

exceptions. These may be due either to an unusual fit or absence of fit between certain toxins and antitoxins, which affects the dilution

TABLE III

Dilution indices for the sera shown in table II, referred to the dilution characteristics of each toxin

Serum	Dilution Indices against toxin					Average dilution Index
	A	B	C	D	E	
1	1·159	1·198	1·152	1·199	1·288	1·199
2	1·179	1·176	1·175	1·199	1·196	1·185
3	1·100	1·077	1·105	1·127	1·196	1·121
4	1·100	1·044	1·128	1·079	1·091	1·088
5	1·022	1·033	1·116	1·067	1·117	1·071
6	1·041	0·967	1·152	1·055	1·064	1·056
7	1·012	1·154	0·975	1·007	0·907	1·011.
8	1·071	1·011	0·987	0·971	1·012	1·010
9	0·973	1·088	0·975	1·007	0·972	1·003
10	0·953	1·033	0·987	0·983	0·854	0·962
11	0·953	0·912	0·881	0·935	0·907	0·918
12	0·884	0·879	0·905	0·887	0·920	0·895
13	0·756	0·747	0·693	0·755	0·775	0·745
14	0·806	0·681	0·776	0·731	0·696	0·738
Dilution characteristic	1·018	0·910	0·851	0·834	0·761	

TABLE IV

Percentage differences between each dilution index referred to serum 12 and the average for the 5 indices determined against different toxins

Toxin		A	B	C	D	E	Average percentage difference
Serum	Average Index	Percentage difference from average Index					
1	1·340	−2·2	+1·7	−5·0	+0·8	+4·5	2·8
2	1·324	+0·7	+1·1	−1·9	+2·0	−1·8	1·5
3	1·252	−0·6	−2·2	−2·5	+1·4	+3·8	2·1
4	1·216	+2·3	−2·3	+2·5	0	−2·5	1·9
5	1·197	−3·4	−1·8	+3·1	+0·5	+1·4	2·0
6	1·179	−0·1	−6·7	+8·0	+0·8	−1·9	3·5
7	1·131	+1·1	+16·1	−4·7	+0·4	−12·8	7·0
8	1·129	+7·3	+1·9	−3·4	−3·0	−2·6	3·6
9	1·122	−1·9	+10·3	−3·9	+1·2	−5·8	4·6
10	1·076	+0·2	+9·2	+1·4	+3·0	−13·7	5·5
11	1·026	+5·1	+1·2	−5·1	+2·7	−3·9	3·6
12	1·000	...	...	...	...	...	...
13	0·833	+2·8	+2·0	−8·0	+2·2	+1·2	3·2
14	0·825	+10·4	−6·0	+3·9	−0·1	−8·2	5·7
Average difference		2·9	4·8	4·1	1·4	4·9	

ratio possibly by influencing the length of range of reactions about the neutral point, thus making the reading of reactions difficult.

The end-point accepted in these determinations was that halfway between the least amount of serum at which no reaction was obtained and the greatest amount at which a definite positive was shown. It is possible that with a different end-point these discrepancies might not have appeared. It was found in practice that toxin B was not a good test toxin : of the 14 sera tested 6 gave long-range results at the Lr/1000 level. Reactions produced by toxin E were diffuse and difficult to read. Of the antitoxins, serum 7 gave irregular results, the dilution index being 16·1 per cent. discrepant in one direction with toxin B and 12·8 per cent. discrepant in the other direction with toxin E : sera 9, 10 and 14 all gave one result which was more than 10 per cent. from the average value. Inspection of the individual tests does not suggest any obvious explanation of these discrepancies, but it should be noted that 4 of these 5 values involved toxins B and E which were found in practice to be the least suitable test toxins for use at the Lr/1000 level: the average error in determination (table IV) was greatest for these two toxins. Table IV shows that there is a certain similarity in the behaviour of sera 7, 9 and 10. Against toxin B, the percentage differences from the average indices were respectively 16·1, 10·3, and 9·2, all these values being higher than the average. Against toxin E the figures were 12·8, 5·8 and 13·7 per cent., below the average. This result seems to suggest differences in goodness of fit. With toxin D, the average deviation was very low, only 1·4 per cent., and all the values against this toxin were within 3 per cent. For general testing purposes a short-growth toxin of this kind is usually very satisfactory.

All 30 values for sera 1-5 and serum 12 were within 5 per cent., and 80 per cent. were within 2·5 per cent., the average being 1·7 per cent. This fact again suggests the influence of length of range of reactions, because in general a more clear-cut end-point is obtained in the titration of antitoxins of good avidity.

Discussion

It would appear, therefore, that very accurate estimations of dilution ratio can be made with a chosen toxin, and these ratios should be referred to a specially selected antitoxin as standard. The toxin used should be such that fairly clear-cut end-points are obtained with most sera at the Lr/1000 level. The antitoxin chosen as standard should not be very non-avid, because such sera tend to give long-range reactions and results may be influenced by the end-point accepted.

The figure shows that there is a rough correlation between serum ratio and dilution ratio, but that sera 2 and 7 are definitely discrepant. Serum 7 was bled from a poorly-responding horse after 4 weeks' immunisation. It has often been noticed at these laboratories that many horses produce antitoxin of high serum ratio in the first few

weeks of immunisation, after which the ratio falls to a constant value, usually about 1·0. The results obtained with serum 7 suggest that a high ratio shown in the early stages of immunisation may not necessarily be connected with good avidity. Another but less striking example of the same phenomenon is to be seen in serum 4, which was also taken early in immunisation. Figures for the dilution ratio of this antitoxin are lower than would be expected from consideration of the serum ratio. Serum 2, whose serum ratio of 4·29 was greatly in excess of that for any other serum, was abnormal. The behaviour of specimens from other bleedings of the same horse as serum 2 was described by Barr and Glenny (1938) ; one of these had a serum ratio of 3·14, the other 5·06, so that the ratio altered during later immunisations. The flocculating properties of all samples of serum from this
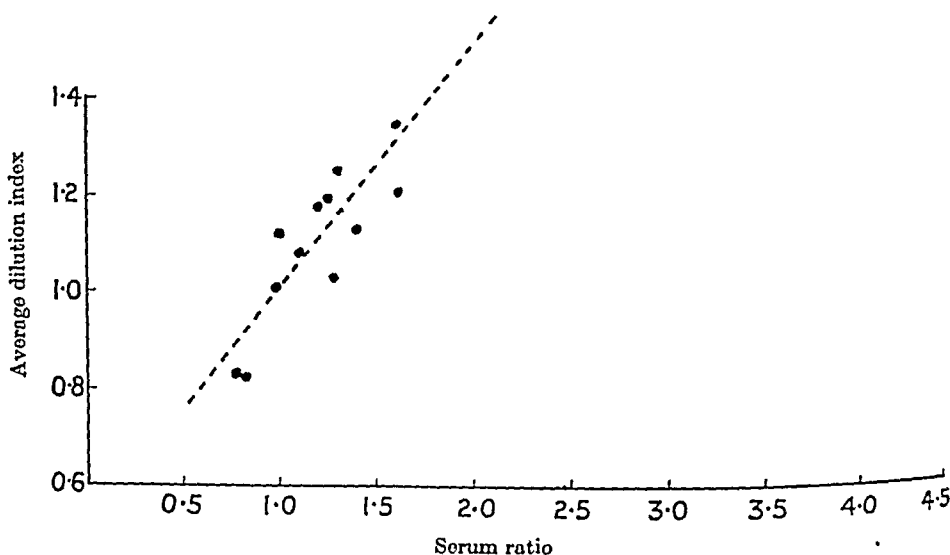
Fig.—Relation of serum ratio and average dilution index of the sera shown in table II.

horse were abnormal in that the value obtained varied according to whether toxins or toxoids were used in its determination. A constant in-vitro value was obtained when determined against 8 different batches of unmodified toxin, but variable values from 9 to 40 per cent. higher when 23 toxoids were used for the test. It was suggested by Barr and Glenny (1931) that all the antitoxin present in sera of high in-vivo/in-vitro ratio may not enter into the flocculation test ; the results obtained by them (1938) lent support to this suggestion and indicated that the proportion failing to flocculate in this particular serum was reduced when toxoids rather than toxins were used. It also appears probable, as they suggested from examination of ammonium sulphate fractions, that sera contain both non-flocculating and non-avid antitoxin, because the serum ratio of successive fractions showed a progressive decrease from 18·9 to 0·86. All sera, therefore,

and more especially those with an abnormally high serum ratio, may contain both non-flocculating and non-avid antitoxin, the proportions of which differ from one serum to another. The peculiar flocculating properties of serum 2 suggest the presence of a considerable amount of non-flocculating antitoxin, and also that the flocculation value of this serum as determined against toxins, though constant, is definitely misleading when considered from the standpoint of serum ratio and avidity.

It is also possible that toxins are not homogeneous, and may consist of molecules possessing a range of affinities for antitoxin. Further, the affinity of these for antitoxin may also vary according to the nature of the antitoxin. On the basis of such a supposition, goodness of fit would occur between a toxin and serum composed of components of maximum mutual affinity present in optimum proportions.

In general, sera with a low serum ratio have a low dilution ratio, and exceptions to the correlation only occur when the serum ratio is over about 2·0.

Summary

1. The dilution ratio of 14 sera was measured against each of 5 toxins, prepared from P.W. 8 and *gravis* strains of *C. diphtheriæ* grown for 3, 4 and 10 days.

2. The values of the ratio differed according to the toxin used : the more toxic the toxin, in terms of number of M.R.D. per Lr dose, the lower the dilution ratio. Against a short-growth *gravis* toxin all sera appeared non-avid, and the average dilution ratio was 25 per cent. less when measured against this toxin than when measured against a long-growth P.W. 8 toxin.

3. The dilution ratios of most sera referred to that of a chosen standard serum (= 1·00 for each toxin used) remained constant within the limits of error, whatever toxin was used. A few exceptions suggest that there may be variation in goodness of fit between certain toxins and antitoxins.

4. One particular toxin gave values of the ratio for the 14 sera, all of which were within 3 per cent. of the average ratios against the 5 toxins. This was a 4-day-growth preparation and such short-growth toxins are usually very satisfactory for use in general testing.

5. A rough correlation existed between serum ratio and dilution ratio. Exceptions were antitoxins whose serum ratio exceeded 2·0.

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LIPOMATOUS PSEUDOHYPERTROPHY OF THE PANCREAS WITH COMPLETE ABSENCE OF EXOCRINE TISSUE

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(Plates XXIV-XXVI)

Hantelmann (1931) described a case of lipomatous pseudohypertrophy of the pancreas in a 9-year-old boy who died from Hodgkin's disease with amyloidosis. The · pancreas was considerably enlarged but of normal shape. Microscopically it consisted of adipose tissue with larger or smaller islets of Langerhans which gave the appearance of being somewhat compressed by the surrounding fat. Only small " rests " of exocrine glands were found, occupying an area 1·5 mm. in diameter. The patient had not presented any symptoms of diabetes or other signs indicative of pancreatic insufficiency. Hantelmann was unable to find more than two analogous cases in the literature.

Rössle (1921) had previously described similar changes in the pancreas of a 12-year-old boy who died of sepsis originating from cellulitis of the neck. Except for slight abdominal pain and some polydipsia and polyuria during the terminal phase of his illness there were no symptoms which could possibly have been due to pancreatic insufficiency. Urine obtained *post mortem* was free from sugar ; there was no information about the blood sugar or sugar in the urine when the patient was alive.

Gross (1926, cited by Hantelmann) reported a case of sprue in a 19-months-old infant with severe wasting (wt. 3500 g.) but no glycosuria, who died of pneumonia. At autopsy, changes were found in the pancreas similar to those in the cases of Hantelmann and Rössle.

The Index Medicus up to the year 1946 refers only to the three cases mentioned.

Case Report

Clinical history

The patient, a 9-months-old boy, was born at full term on 11th May 1946. The weight at birth was 3400 g., the length 48 cm. The family was healthy and the pregnancy had been uneventful. The infant grew normally for the first 7 months of life and at six months weighed 6100 g. At seven months he contracted a conjunctivitis and nasopharyngeal catarrh, which was treated with sulphathiazole, 4·5 g. being administered over a period of 3 days ; no improvement resulted. An exanthema resembling urticaria developed on the face, and medication was therefore discontinued. Three weeks later a

pædiatrician prescribed penicillin ointment and eggs and bananas were added to the diet. Within three days the infant had recovered completely. After this he was lively and slept well but refused food. He lost weight and at 9 months weighed only 5530 g. Stools were passed three times a day. They were soft and pulpy and had a foul odour, and the mother noticed globules of fat floating on the water in the chamber-pot. Blood and mucus had not been observed. The patient was admitted to the pædiatric clinic, Rikshospitalet, with the diagnosis "dystrophia."

Clinical examination (6th February 1947). The patient was a poorly nourished 9-months-old boy with flabby, wrinkled buttocks. He was unable to sit upright and showed slight signs of rickets. Temperature 36·7° C., pulse 160, respirations normal. Abdomen large and distended, with a very prominent umbilical hernia. Liver and spleen just palpable. No abnormality discovered in chest, external genitals, rectum, ears, nose or urine. The throat showed a slight pharyngitis.

Examination of the blood. A blood count on 7th February showed R.B.C. 3·8 million, Hb. 90 per cent., white cells 5200 (lymphocytes 85 per cent.), thrombocytes 217,000. Later leucocyte counts on 10th March and 10th April showed 5100 and 7600 respectively, with lymphocytes 82 and 89 per cent. The blood sedimentation rate (Landau's micro-method) was 11 mm. per hour on 7th February, 4 mm. on 10th March and 2 mm. on 10th April. Hb. values ranged from 110 to 78. Blood calcium values were 9·09, 9·49 and 10·79 mg. per 100 c.c. Prothrombin time 29 (22) and 29 (25) seconds. Serum-iron 132 gamma per 100 c.c. Wassermann reaction negative. The blood-sugar curve, tested twice, was normal or possibly slightly flattened.

X-ray examination of the wrists at the time of admission revealed slight signs of rickets. One month later these signs had disappeared. X-ray examination of the intestines disclosed a somewhat enlarged sigmoid but otherwise normal conditions. A chest roentgenogram was normal.

During the child's stay in hospital the stools appeared greyish and fatty and had a foul odour. They were passed 1-5, usually 2-3, times a day. The content of fat in the dried stools was 44·4 per cent. Microscopically there was an abundance of neutral fat and some fatty-acid crystals. No iodophil starch granules were found.

The condition was diagnosed as cœliac disease and dietary treatment was instituted. From 28th March amino acids ("Aminosol" 10 g. per day) were added to the diet and the weight gradually increased, so that on 8th May the infant weighed 5900 g. However, he seemed loath to take his food and usually spat it out. On 10th May, just as he had taken some of his dinner, he suddenly collapsed, with cessation of respiration and cyanosis. The hypopharynx and larynx were immediately aspirated and some food was brought up. Artificial respiration was instituted, together with administration of oxygen and stimulants, but without result. The heart's action continued for 15 minutes until death.

Autopsy

Both glottis and larynx were obstructed by remnants of food, extending 1-2 cm. downwards into the trachea. The remainder of the trachea and the first 1-2 cm. of the main bronchi were free. Further down, both main bronchi and the lobar bronchi were again obstructed by food remnants. Both lungs were œdematous. The bronchial lymph nodes were soft and slightly enlarged. Liver, gall-bladder and biliary tracts weighed 220 g., spleen 28 g., adrenals 2 g. and kidneys 44 g. All these organs appeared to be normal. In the distal

part of the ileum there was moderate enlargement of the Peyer's patches, and in the cæcum and transverse colon numerous enlarged lymph follicles were found. The rest of the intestinal tract and the stomach were normal. The mesenteric lymph nodes were considerably enlarged and partly arranged in groups. They seemed to be firmer than normal. Pelvic organs and brain (800 g.) and meninges normal.

The pancreas (21 g.) was enlarged, 7 g. being considered normal for this age (Rössle and Roulet, 1932). Its shape was normal and it felt firm. The cut surface was pale, with poorly defined lobulation.

Microscopic examination

There is slight fatty change in the liver, which is otherwise normal. The lung alveoli contain much fluid. There is a considerable increase of the lymphoid tissue of the ascending colon. The mesenteric lymph nodes are œdematous but otherwise normal. The spleen, kidneys, adrenals, thymus, heart, thyroid and stomach are normal.

Pancreas. Sections from the head, two different parts of the body, and the tail of the pancreas show identical pictures. The organ is covered by a thin fibrous capsule from which fine fibrous septa extend into the underlying tissue. Scattered areas of young small-celled fatty (adipose) tissue are seen external to this capsule (fig. 1). Inside it there is found a highly differentiated mature adipose tissue divided into lobules by fine fibrous strands and containing sparsely scattered, more or less complete islets of Langerhans (figs. 2 and 3). These islets are partly discrete, partly arranged in conglomerates. Most of them do not show the usual compact structure. The cells are smaller, slightly shrunken and dense, probably because of post-mortem changes and the technique employed. Some of the islets are small and atrophied and contain hyaline masses which present a deep red border zone and a pale discoloured central area when stained with eosin. In some places large numbers of nuclei occupy the periphery of these masses (fig. 4), so that the whole picture resembles that of large giant cells. The number of islets is apparently normal or slightly reduced. The cytoplasm of the islet cells contains granules which stain orange to red by Mallory's stain. Alpha and beta cells cannot be clearly differentiated (44 hours *post mortem*). The exocrine glandular tissue is completely replaced by the fatty tissue. The persisting connective tissue contains ducts which correspond to the large excretory ducts of the normal pancreas (fig. 5). No Laguesse-Bensley ducts can be distinguished with the mucicarmine stain. The connective tissue also contains Pacinian bodies and small nerves. The impression given is thus of adipose tissue with remnants of excretory ducts, total absence of acinar tissue and probably a normal or somewhat reduced number of persisting islets.

These pancreatic changes correspond closely with those of the three previously reported cases, especially that of Gross. He too

discovered these changes in a small child with symptoms of cœliac disease.

Because of the increase in weight and size of the pancreas Hantelmann suggested that the condition be known as lipomatous pseudohypertrophy of the pancreas.

DISCUSSION

The ætiology of the disease *is unknown. The age incidence and the complete or almost complete absence of acini but persistence of the islets of Langerhans suggest that the condition is due to a congenital aplasia of the exocrine portion of the gland.* It will be recalled that the pancreas arises from two separate primordia, a smaller ventral and a larger dorsal, both appearing in the 4th week of fœtal life (the 3-mm. stage) as two diverticula of the duodenal endoderm. The dorsal primordium appears at a somewhat earlier stage and grows more rapidly than the ventral and gives rise to the body and tail. *The head and a small part of the body are formed from the ventral primordium.* The two primordia fuse in the 6th week of fœtal life (the 12-mm. stage). The endodermal pancreatic diverticula increase in length and extend in the mesenchymal tissue. From the end of these sacs there is an outgrowth of epithelial sprouts which become canalised to form the excretory ducts. The acini are formed by further epithelial sprouts from these ducts. *The islets are formed in a similar way, but in the 3rd fœtal month they become separated from the ducts to form isolated groups of cells in which secretion granules can be recognised.* Occasionally the connecting ducts between the islets and the ducts themselves may persist as the Laguesse-Bensley ducts (Torgersen, 1948). *Proteolytic pancreatic enzymes are produced by the acini from the 5th fœtal month* (Hamilton *et al.*, 1945).

From an embryological point of view it is quite possible for aplasia of the exocrine apparatus or of the islets to occur as a result of lack of differentiation during the sprouting stage.

Complete absence of the pancreas is seen only in connection with extreme malformations of the fœtus (Kaufmann, 1931). Defective development of the dorsal pancreatic primordium will result in a pancreas which merely consists of the head, with or without a short tail, as described by Kriss (1927), who, besides two cases of his own, found four others reported. In these cases the distribution of the acini and islets and the histological picture generally were normal. Three of the patients had diabetes. Congenital complete absence of islets has also been reported (Moore, 1944 ; Lasowski, cited by Hantelmann). Lack of differentiation during the stage of sprouting is thus shown to occur. Congenital absence of acini, though so far not described, might explain lipomatous pseudohypertrophy. This theory is supported by the fact that the condition occurs in infants and children and that the lack of acini is complete or almost complete.

LIPOMATOSIS OF PANCREAS

FIG. 1.—The lipomatous tissue which replaces the pancreas consists of large " mature " cells. It contrasts sharply with the smaller-celled embryonal fatty tissue, from which it is separated by the capsule of the pancreas. Hæmatoxylin and eosin. × 60.
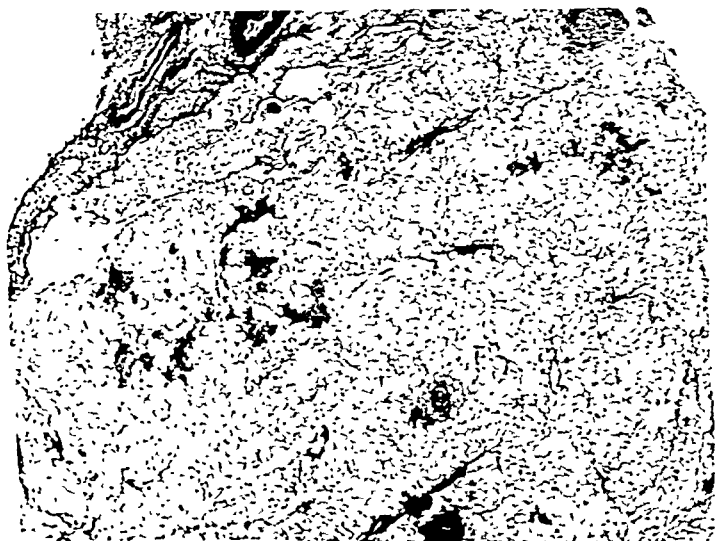
FIG. 2. —Groups of islets of Langerhans are irregularly scattered throughout the fatty tissue. Note complete absence of acini. × 15.

LIPOMATOSIS OF PANCREAS

FIG 3.—An aggregation of islets of Langerhans is shown surrounded by large celled fatty tissue. To the right is seen one of the ducts of the pancreas surrounded by a zone of densely collagenous connective tissue. Bielschowsky's silver stain. × 100.
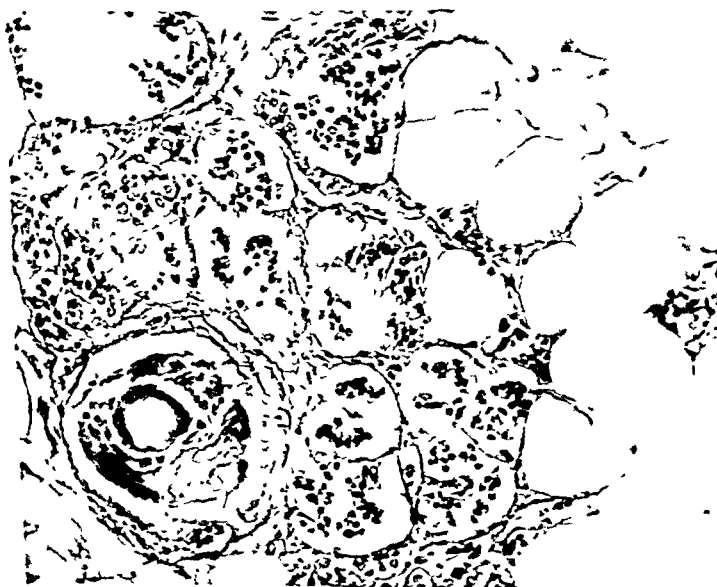
FIG 4 A group of islets of Langerhans is shown, of which one of the islets presents degenerative changes and contains a hyaline mass surrounded by a ring of nuclei, the whole simulating a giant cell. Bielschowsky's silver stain. × 200.

Against the theory are the facts that the organ is of normal shape and that its weight is above normal, whereas with congenital absence of islets the weight is reduced.

There are also examples of partial lipomatosis of the pancreas with partial reduction in the number of acini. This condition usually occurs in elderly individuals and is frequently associated with diabetes and general obesity. Hantelmann has collected 9 such cases from the literature, and others have been reported by Baló (1929). The fact that the present case gave rise to no symptoms until the infant was 7 months old does not necessarily indicate that the disease was acquired, since symptoms of pancreatic insufficiency may well be missing, as in the cases reported by Hantelmann and Rössle.

Assuming that the disease is acquired, the condition may be explained in one or other of the following ways.

1. By occlusion of the excretory ducts. If these ducts be ligated or obstructed there is rapid disintegration of the acini whereas the islets are unaffected. A mechanism like this may possibly be involved in fibrocystic disease of the pancreas, a condition in which there is atrophy of the lining epithelium of the ducts, which are dilated and filled with dehydrated secretion. The acini are more or less atrophic whereas the islets remain unaffected. There is a definite increase of the interlobar and interacinar connective tissue, which is often infiltrated with leukocytes. On the other hand no fatty metamorphosis is seen. In the present case, no obstruction of the excretory ducts could be demonstrated, but systematic examination by means of serial sections of the whole pancreas was not carried out.

2. By injury to the parenchyma caused by infection or toxico-allergic agents resulting in an adipose metamorphosis. Supporting this view is Rössle's case, which showed small nests of acinar tissue with signs of disintegration and the accumulation of various types of lymphoid round-cells, as well as slight signs of regeneration in the form of mitotic figures in the acinar cells and slight sprouting. Apolant (1913), too, has recorded lipomatosis with almost complete disappearance of the exocrine glandular parenchyma in three mice which were used for chemo-therapeutic experiments. Various remedies were used, but their nature is not stated. In the case here reported sulphathiazole medication had been used, as a result of which an urticaria developed, and the onset of the disease can be dated from this time.

Escape of the islets may have resulted from their being more resistant than the acini. Examples of this can be found in the literature, *e.g.* in fibrocystic disease of the pancreas. Further, in congenital syphilitic fibrosis of the pancreas the glandular parenchyma is poorly developed, whereas the islets are normal and may even be more numerous than usual. In carcinoma of the pancreas, too, the islets are well preserved (Bakken, 1947; personal communication). Hantelmann thinks that the condition is probably acquired and

LIPOMATOSIS OF PANCREAS
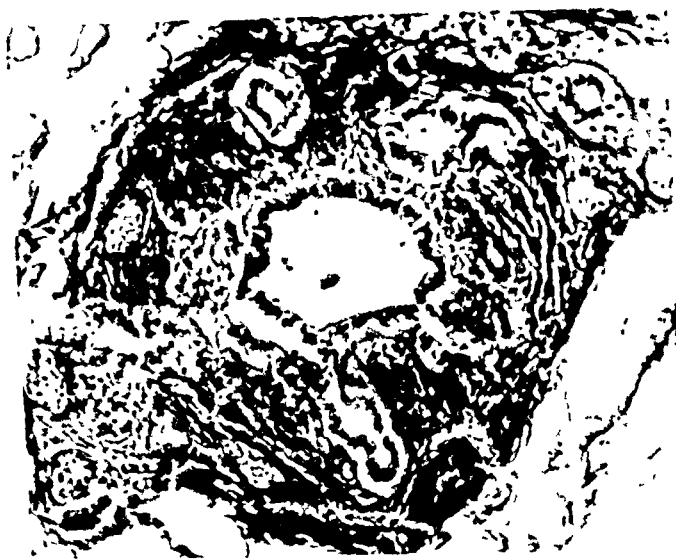
FIG. 5.—One large and several small pancreatic ducts are shown embedded in inter-lobular connective tissue. Islets of Langerhans are seen at the periphery. Van Gieson. ×165.

FIG. 6.—Two different parts of the pancreas from case 2. In the midst of the fatty tissue pancreatic ducts and small "rests" of pancreatic parenchyma are seen. Hæmatoxylin and eosin. ×2.
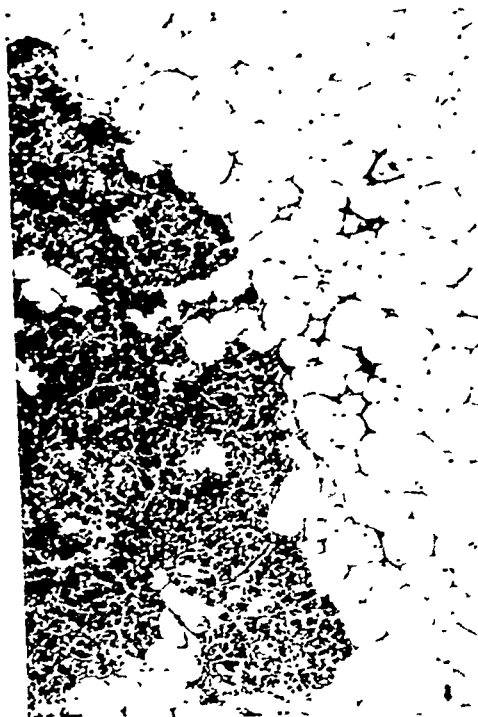
FIG. 7.—A "rest" of pancreatic parenchyma is shown, containing a single islet of Langerhans, the whole surrounded by fatty tissue, in which there is slight focal infiltration by lymphocytes, plasma cells and myeloblasts (case 2). Hæmatoxylin and eosin. ×60.

caused by chronic infection of toxic agents of unknown origin. Most likely this applies to the case now reported. The possibility of a toxico-allergic reaction caused by the sulphathiazole cannot be excluded, but neither can congenital aplasia of the exocrine apparatus.

A case recently observed at the Institute of Pathology of the Rikshospitalet, Oslo, is of great interest in this connection.

A woman 69 years old, who had previously been in good health, died after having had symptoms of myelogenous leukæmia for four years. She had been treated with iron, arsenicals and hydrochloric acid, and had also had several blood transfusions and injections of liver extracts.

The autopsy, performed 6 hours *post mortem*, revealed a pancreas of normal shape and 70 g. weight, the average in the adult being 58.65 g. (Rössle and Roulet). She had had no symptoms which might suggest pancreatic insufficiency and there had been no evidence of diabetes. The gross appearance on section was of fatty tissue in which small rests of pancreatic parenchyma could be demonstrated (fig. 6). Microscopically a highly differentiated mature adipose tissue is seen in which are scattered small irregular areas consisting of normal-looking islets of Langerhans surrounded by acinar tissue showing signs of atrophy and degeneration, especially at the periphery. In some places isolated islets of Langerhans are surrounded by fatty tissue. In the connective as well as the adipose tissue there is infiltration by lymphocytes, plasma cells and myelo-blasts, partly diffuse, partly focal. Around the isolated islets the focal arrangement is pronounced. The connective tissue also contains excretory ducts of normal appearance with empty lumina (fig. 7). The liver weighed 1720 g. and is normal microscopically.

This case represents a lipomatosis of the pancreas with incomplete disappearance of the exocrine tissue but with well-preserved islets of Langerhans. The weight of the organ is somewhat above the average but within the normal range. It cannot therefore be called pseudo-hypertrophy. The age of the patient also differs from that in the other cases of this lesion and the pathological findings are those of the partial lipomatosis described by Hantelmann. On the other hand the very considerable loss of acinar tissue is striking. Assuming, therefore, that the picture indicates a process still continuing, the case might be regarded as one of early lipomatous pseudohypertrophy, with the possibility of a further increase in weight, resulting in further destruction of the acini and a progressive adipose metamorphosis. The lymphocytes and plasma cells may have appeared in the course of an infectious or allergic condition, or may be secondary invaders following the general atrophy of the exocrine apparatus.

Symptomatology

Cessation of the exocrine function of the pancreas may give rise to cœliac disease, characterised by an insidious onset and wasting, especially of the buttocks, groins and axillary folds and least of the face. The abdomen is distended. The stools are voluminous, soft and pulpy; they are of a light colour and have a foul odour. There is a flattened blood-sugar curve and the absorption of fat is impaired. There is abnormal bowel mobility, and X-ray examination shows clotting of the barium contrast in the small intestine (Mitchell and Nelson, 1946). In some cases none of these symptoms appear, even

PLATE XXVI

LIPOMATOSIS OF PANCREAS

FIG. 5.—One large and several small pancreatic ducts are shown embedded in interlobular connective tissue. Islets of Langerhans are seen at the periphery. Van Gieson. × 165.

FIG 6.—Two different parts of the pancreas from case 2. In the midst of the fatty tissue pancreatic ducts and small "rests" of pancreatic parenchyma are seen. Hæmatoxylin and eosin. × 2.

FIG 7.—A "rest" of pancreatic parenchyma is shown, containing a single islet of Langerhans, the whole surrounded by fatty tissue, in which there is slight focal infiltration by lymphocytes, plasma cells and myeloblasts (case 2). Hæmatoxylin and eosin. × 60.

where microscopical examination of the pancreas shows that no external secretion was possible (Hantelmann, 1931 : Blackfan and May, 1938). In the new-born, occlusion of the pancreatic duct may cause dehydration of the meconium, resulting in a mechanical ileus (Kornbluth and Otani, 1929).

Diagnosis

The condition does not always produce symptoms, as in the cases reported by Hantelmann and by Rössle. On the other hand it may produce symptoms of coeliac disease, as in the present case and that reported by Gross. In order to determine whether a case is one of coeliac disease, which is of a benign nature and usually followed by complete recovery, or whether there is a more grave impairment of the exocrine pancreatic function, it is necessary to examine the duodenal fluid for pancreatic enzymes. In the usual " benign " coeliac disease these enzymes appear in normal amounts, but are missing in fibrocystic disease of the pancreas and presumably also in the lesion here described. Such an examination has not been carried out in any of the four cases so far reported.

Therapy. The condition is treated as one of coeliac disease if and when the proper symptoms are present.

Prognosis. Nothing definite is known on this point. The fact that two of the cases were 9 and 12 years old respectively, and also that there was clinical improvement and increase in weight in the present instance during the terminal phase may indicate that the prognosis is not invariably unfavourable.

SUMMARY

A male infant, born at full term of healthy parents, developed symptoms of coeliac disease and refused to take food following a catarrhal infection at the age of 7 months. Prior to this the child thrived well and gained weight uniformly. Improvement followed dietetic treatment and Aminosol medication. At the age of one year the child died from suffocation while being fed. Autopsy revealed enlargement of the pancreas, which was three times the normal weight for the age. The pancreas, which was normal in shape, was composed of highly differentiated mature adipose tissue containing well-preserved islets of Langerhans in normal or slightly reduced numbers. Remnants of excretory ducts could be seen, but there was complete absence of exocrine tissue. No Laguesse-Bensley ducts could be found. Apart from slight fatty degeneration of the liver, the autopsy revealed only the changes in the respiratory organs caused by suffocation. The changes in the pancreas are identical with those seen in lipomatous pseudohypertrophy of this organ, a rare condition described by Hantelmann in 1931. The present case is the fourth on record. The ætiology is unknown, but the disease is most probably acquired and not congenital, the result of injury to the pancreatic parenchyma by infection or toxico-allergic agents, leading to acinar atrophy and adipose metamorphosis. In the present case sulphathiazole may possibly have been the causative agent. The condition may progress without symptoms, or it may produce symptoms of coeliac disease.

A case presenting partial lipomatosis of the pancreas, possibly an early stage of the same lesion, is briefly referred to.

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THE EFFECT OF TRAUMA ON THE PENTOSE CONTENT OF THE PLASMA IN ANIMALS

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THE demonstration that the shock-inducing action of muscle extracts is due to adenosine triphosphate (Green, 1943 ; Bielschowsky and Green, 1943) raised the possibility that this nucleotide or its metabolic products might be concerned in that general response of the body to injury commonly spoken of as traumatic shock. Several attempts have been made to investigate this possibility, one of which was the study of the effects of trauma on the distribution of nucleotides in the body. Many experiments have now been performed to determine the effects of various forms of injury on the distribution of these compounds in the blood stream. Unfortunately nucleotides such as adenosine triphosphate (ATP) cannot be estimated directly and alterations in the distribution of nucleotide must be assessed from the variations in the concentration of the different fractions of the molecule, *i.e.* from determinations of phosphate, adenosine and pentose.

In common with others (Beall *et al.*, 1941 ; Blalock and Duncan, 1942 ; Duncan, 1943 ; Mylon and Winternitz, 1945, 1946 ; McShan *et al.*, 1945 ; Darmady, 1946-47) we have found that trauma leads to an increase in the inorganic phosphate level in the blood of both man (Green *et al.*, 1949) and animals (Stoner and Green, 1944). There are many possible sources of this excess inorganic phosphate and, as it is only an end product of nucleotide catabolism, these results give only very indirect evidence of a disturbance in nucleotide metabolism. Attempts to determine the blood level of the acid labile " 7 min." phosphorus, which should give more direct information, have led to difficulties both in technique and in interpretation. The results obtained were not very satisfactory (Stoner and Green, 1944) although in some rabbits trauma appeared to raise the " 7 min." P content of the blood.

More satisfactory evidence was obtained from determinations of the adenosine equivalent. In the rabbit as in man, various forms of injury are accompanied by statistically significant increases in the adenosine equivalent of whole blood (Stoner and Green, 1944, 1945a and b, 1948).

A serious experimental difficulty arises from the rapidity with which

extracellular nucleotide is decomposed by dephosphorylation and deamination to a stage at which it can no longer be detected by determinations of the " 7 min." P and the adenosine equivalent. This process is slower in the rabbit (Ostern and Mann, 1933) and in man and it is only in these species that alterations in the adenosine equivalent have been detected after injury or limb ischæmia or, in the case of the rabbit, after the intramuscular injection of fatal doses of ATP. This difficulty directed our attention to a lower and more stable metabolite of ATP, namely the pentose, d-ribose. It was hoped by studying the effect of trauma on the plasma levels of this compound to confirm chemically the conclusions reached from the adenosine-equivalent assays and to extend our knowledge of the effects of trauma in the rat, where no changes in the adenosine equivalent could be detected. This object has been achieved and our results showing the effect of injury on the plasma-pentose level provide further evidence in support of the view that trauma is associated with an alteration in the nucleotide distribution of the body.

METHODS

Limb ischæmia was produced in the rat by a metal clamp (Green, 1943) and in the rabbit by the method of Bywaters and Popják (1942). In both cases the occlusion was applied under ether anæsthesia. Direct muscle trauma in the rat was produced by giving 5 blows to each thigh of an etherised rat with a 200 g. weight falling through 75 cm. Scalds were produced by immersion of the depilated hind quarters of the etherised rat in water at 70° C. for 15 sec. Blood samples were obtained by cardiac puncture and the effects of sampling and anæsthesia were determined in control series of animals. Heparin was used as an anti-coagulant and the plasma pentose, both total and phosphorylated, was determined by a modification of Mejbaum's (1939) method (Green, Bielschowsky and Stoner, unpublished *). All the animals were starved for 18 hr. before use and removed from contact with sawdust for the same period. ATP was injected as the Mg salt prepared from BaATP (Boots).

RESULTS

Control series

The results obtained in the control series (tables I and II) show that, under our experimental conditions, cardiac puncture and short ether anæsthesia had no significant effect on the plasma concentration of either the total pentose or its phosphorylated fraction (pentose P).

Effect of the injection of adenosine triphosphate

In the light of our experience with the adenosine assays it was important to determine the effect of injected ATP on the plasma-pentose level before proceeding further. In the rat, the injection of

TABLE I

Effect of cardiac puncture on the total plasma pentose of the rat. Rats bled by cardiac puncture under ether anæsthesia. Second cardiac puncture 6 hours after first

Rat no	Total plasma pentose (mg per 100 ml)	
	At 0 hr.	At 6 hr.
47	5·32	5·29 *
48	5·41	5·21
49	5·44 *	5·41 *
50	5·13 †	5·00 †
51	5·05	5·26 *

* Slight hæmolysis (faint pink).
† Hæmolysis (pink).

TABLE II

Effect of repeated blood sampling by cardiac puncture on the plasma pentose of the rabbit. Each blood sample 4 ml.

Rabbit no	Pentose level of plasma (mg. per 100 ml)							
	At 0 hr.		At 4¼ hr.		At 5¼ hr.		At 5¾ hr.	
	Total	P	Total	P	Total	P	Total	P
28	4·83	3·63	4·76	3·45	4·81	3·45	...	...
29	4·24	2·34	4·26	2·34	4·24	2·35	...	...
30	4·65	2·36	...	...	4·65	2·35	4·61	2·34
33 *	3·92	2·38	3·69	1·99	3·74	1·98	...	...
34 *	3·87	1·87	3·77	1·92	3·87	2·09	...	...
35 *	3·51	2·19	...	...	3·45	2·22	3·33	2·22

* These animals were given a short light ether anæsthesia after the first specimen had been withdrawn.

P = phosphorylated fraction.

TABLE III

Effect of intramuscular injection of 50 mg. MgATP per 100 g. body wt. on the plasma pentose of the rat

Rat no	Total plasma pentose (mg per 100 ml)	
	Before injection	1 hr. after injection
52	4·76 *	7·14 †
53	5·41	9·08 *
54	5·49	8·48 *
55	5·35	8·34 *
56	5·41	7·68 *
57	5·82	7·88
58	5·78	6·26 †

* Slight hæmolysis (faint pink).
† Hæmolysis (pink).

a fatal dose of MgATP (50 mg. per 100 g. body wt. I.M.) gave a striking and significant increase (P = 0·01) in the total pentose content of the plasma examined 1 hr. after the injection (table III).   In the rabbit also the intramuscular injection of fatal doses of MgATP (600 mg. per kg. body wt.) gave increases in the plasma-pentose level of the same order as those which occurred in the rat.   Since alterations in plasma-pentose concentration can be detected after fatal doses of ATP in both the rat and the rabbit, the method would appear to be suitable for investigating disturbances in nucleotide metabolism.

Effect of limb ischæmia

The effect of a 3-4-hr. period of bilateral hind-limb ischæmia on the plasma-pentose concentration in the rabbit is shown in table IV.

TABLE IV

Effect of ischæmia of both hind limbs on the plasma pentose of the rabbit

Rabbit no.	Period of Ischæmia (hr.)	Plasma pentose (mg. per 100 ml.)								
		Initial		Just before release of tourniquets		After release of tourniquets					
						At 0·5 hr.		At 1·0 hr.		At 1·5 hr.	
		Total	P	Total	P	Total	P	Total	P	Total	P
71	3	4·00	2·27	3·57	2·45 *	...	...	...	...	...	...
72	3	6·21	3·79	9·09	4·63 †	...	...	...	...	11·11	8·62 *
95	3¾	4·37	1·75	...	...	...	...	8·20	6·41	...	...
96	3¾	4·61	2·11	...	...	6·09	4·90 †	...	...	...	...
59	4	6·55	4·35	7·60	3·47	8·00	3·40	...	...	...	...
60	4	5·52	3·25	...	...	6·85	4·77	...	...	...	...
75	4	6·66	2·45	...	...	...	...	...	...	11·11	8·62 *
76	4	4·76	2·43	...	...	...	...	...	...	5·05	3·09
77	4	5·18	3·13	...	...	...	...	...	...	9·43	5·96
78	4	6·45	3·85	...	...	...	...	...	...	9·26	5·62 †
79	4	4·85	2·26	...	...	11·62	8·77	...	...	...	...
80	4	5·88	3·40	...	...	9·52	4·81	...	...	...	...
81	4	5·85	2·57	...	...	10·30	5·75	...	...	...	...
86	4	4·79	2·78	...	...	...	...	4·72	2·79	...	...
121	4	5·13	4·13	...	...	...	...	5·41	4·59	...	...
122	4	5·34	4·00	...	...	...	...	7·04	4·90	...	...

* Slight hæmolysis (faint pink).
† Hæmolysis (pink).
P = phosphorylated fraction.

These results are very different from those obtained in the controls (table II), for in all except 2 of the 16 animals this form of trauma led to an increase in the level of both the total pentose and its phosphorylated fraction (pentose P).   From a statistical point of view the increases in both fractions are highly significant (P = 0·01) if all the values obtained after the release of the tourniquets are considered together.

The effect of unilateral hind-limb ischæmia on the plasma-pentose levels in the rabbit was also studied (table V). In these experiments

TABLE V

Effect of ischæmia of one hind limb of the rabbit on the plasma pentose in the blood draining from that limb

Rabbit no	Plasma pentose (mg. per 100 ml.)						Time after release of tourniquet when specimen taken (min.)
	Pre-ischæmic specimen (cardiac puncture)		Normal limb		Ischæmic limb		
	Total	Phosphorylated fraction	Total	Phosphorylated fraction	Total	Phosphorylated fraction	
41 *	6·66	3·23	6·66	3·38	6·66	3·16	Control : no ischæmia
39 †	5·15	2·33	7·69	3·79	7·07	5·00	15
40 †	4·47	2·67	6·66	4·27	7·69	4·95	25
42 ‡	8·47	3·20	9·09 ‖	4·17 ‖	11·11	4·35	15
43 ‡	7·41	2·50	...	...	10·87	4·67	60
37 §	...	...	6·25	2·77	6·85	3·03	0

* Control : no ischæmia
† Period of ischæmia 2 hr.
‡ Period of ischæmia 3 hr
§ Period of ischæmia 4 hr.
‖ Slight hæmolysis (faint pink).

the initial blood specimen was withdrawn from the heart by cardiac puncture and subsequent specimens from the femoral veins draining the hind limbs. It was found that, after ischæmia, the plasma-pentose levels, both total and phosphorylated, were higher in the blood coming from the hind limbs than in the initial specimen of heart blood. Furthermore, in the majority of cases the levels in the blood from the injured side tended to be greater than those on the normal side. The control experiment showed that the pentose levels in the femoral-vein blood from the two sides were the same as those in the heart blood.

In these experiments figures are available for the changes in both the total plasma pentose and its phosphorylated fraction. Consideration of these data shows that in 10 of the 22 observations the increase in the pentose P fraction exceeded that in the total pentose fraction and that in 9 of the remaining observations it accounted for more than 50 per cent. of the increase in the total pentose. This is important, because a much more definite chemical meaning can be attached to the pentose P fraction.

In the rat the total pentose concentration was examined immediately before a 4-hr. period of bilateral hind-limb ischæmia and 2 hr. after the release of the occlusion (table VI). Again there was a striking increase, significant at $P = 0.01$, in the pentose content of the specimen taken during the post-ischæmia period compared with the control series (table I).

Effect of direct muscle trauma

Direct muscle trauma in the rat also increased the total pentose concentration of the plasma (table VII). The increase which occurred under these conditions was not quite as great as that produced by

TABLE VI

Effect of bilateral limb ischæmia on the plasma-pentose fraction of the rat. Rats bled by cardiac puncture under ether. Period of ischæmia 4 hours

Rat no.	Total plasma pentose (mg. per 100 ml.)	
	Before ischæmia	2 hr. after release of occlusion
42	5·26	9·52 *
43	5·82	7·69 *
44	5·88	8·33
45	6·25	8·77 *
46	5·13 *	8·85

* Slight hæmolysis (faint pink).

TABLE VII

Effect of direct muscle trauma on the plasma pentose of the rat. Rats bled by cardiac puncture under ether

Rat no.	Total plasma pentose (mg. per 100 ml.)	
	Before trauma	½ hr. after trauma
69	5·48 *	7·35 *
70	5·32 *	5·78 *
71	5·26	7·04
72	4·08	6·41 †

* Slight hæmolysis (faint pink).
† Hæmolysis (pink).

limb ischæmia, being significant only at $P = 0.05$, but it still presented a marked contrast to the changes in the control rats, where the only trauma was cardiac puncture (table I).

Effect of thermal trauma

The changes in the plasma-pentose concentration in rats subjected to scalding were similar to those which occurred after direct muscle trauma, but the presence of gross hæmolysis in the plasma samples made interpretation difficult (*vide infra*).

DISCUSSION

These results show that tissue injury of various types is accompanied by a big increase in the pentose concentration of the plasma of about the same degree in both the rat and the rabbit. As in the case of the

adenosine equivalent (Stoner and Green, 1948) these changes are of a similar order to those produced by fatal doses of ATP.

The chemical significance of the results obtained by our method has been dealt with in a report to the Medical Research Council (see footnote to p. 102). For present purposes it may be said that the total pentose values represent all the pentose present in the plasma, either as free pentose or combined as ribose phosphate, nucleoside or nucleotide. The pentose P fraction represents that portion of the total pentose which is linked to one or more phosphate groups, *e.g.* ribose phosphate and the pentose present in nucleotides. Changes in this latter fraction, the estimation of which is much less affected by the presence of substances such as glucose, are much more significant from our point of view. It is interesting, therefore, to observe that not only are both fractions concerned in the increase after trauma but that the increase in total pentose seems to be largely due to a change in the phosphorylated fraction.

The main difficulty in assessing the biological significance of these results is in determining the part played by hæmolysis in the production of the increase in the plasma-pentose level after trauma. The red cells contain much more pentose than the plasma and in experiments on the blood after trauma hæmolysis is difficult to eliminate, no matter how careful one's technique, since all forms of large-scale tissue injury render the red cells more fragile (Knisely *et al.*, 1945 : Ricca *et al.*, 1945). This is especially true in the rat, particularly after scalding. In the rabbit there appears to be less tendency for hæmolysis to occur after injury, so that the results in this species are more reliable. As far as possible we have excluded the rather numerous experiments in which gross hæmolysis occurred. In an attempt to see how far the observed increases could be accounted for by hæmolysis we have indicated in the various tables the incidence and degree of hæmolysis in the plasma specimens. When the figures shown for the rabbit experiments are studied from this point of view it is obvious that there is no close correlation between the degree of hæmolysis and the increase in the plasma pentose, and that in the majority of experiments the changes cannot be accounted for in this way. The position with regard to the rat figures is less clear, since hæmolysis occurred more frequently in these experiments. In most of the experiments recorded here in which hæmolysis occurred, its degree was only *slight* and there are sufficient experiments without hæmolysis to suggest that at least it is not a major factor in the production of the increase in plasma pentose. A probable exception to this was the result obtained in the rat after scalding, where hæmolysis was so gross as to make it impossible to be certain that it did not play a major part in the effect. Consequently, although we cannot exclude the erythrocyte as a possible source of the excess pentose, we do not consider that it is the major source after most forms of trauma. McShan *et al.* have also reported an increase in the plasma pentose of the rat after

trauma (Noble-Collip drum and limb ischæmia), but although they considered the possibility of hyperglycæmia interfering in their estimations they did not take into account the more important factor of hæmolysis and consequently their results are difficult to evaluate. Hyperglycæmia does occur after trauma of the types used here, but alterations in the glucose content of the plasma of the order seen have been shown not to be a major source of error in the estimations of plasma pentose by the method used in this laboratory.

The main significance of these observations on the effect of trauma on the plasma-pentose level is that they confirm by a chemical method the results of the adenosine equivalent determinations which are dependent upon a biological assay. The agreement is only qualitative; close quantitative agreement between two such dissimilar methods was hardly to be expected, but the qualitative similarity is close. The times of occurrence of the increase in the two fractions are similar, as are also the magnitudes of the increases. In both cases limb ischæmia causes a greater change in the blood level than direct muscle trauma. In both cases the average percentage change in the blood level is of the same order. When all the data available to us are considered, both from our own work and from that of others (Billings and Maegraith, 1938; Cullumbine, 1947; Kalckar and Lowry, 1947), it would seem to be beyond reasonable doubt that trauma is accompanied by an increase in nucleotide metabolites in the blood stream.

Of themselves, the plasma-pentose results do not give much indication of the form in which these metabolites exist, but when these data, especially those relating to the change in the pentose P fraction, are considered in conjunction with what we know of the behaviour of the adenosine equivalent under these conditions, it would seem that a fair proportion of the increase is accounted for by nucleotide, at least of the lower types such as muscle adenylic acid. Whether these substances play any part in the development of the picture of traumatic shock is another question to which no conclusive answer can yet be given.

Summary

The effect of various lethal forms of trauma on the plasma-pentose levels have been investigated in the rabbit and rat.

In the rabbit, limb ischæmia is followed by an increase in the concentration of both the total pentose and its phosphorylated fraction. A similar increase occurs in the rat after limb ischæmia, direct muscle trauma and scalds.

These changes, which are of the same order as those produced by the intramuscular injection of fatal doses of adenosine triphosphate, cannot be accounted for by the method of blood sampling or anæsthesia.

Trauma is usually accompanied by increased fragility of the red cells and the possibility that the excess pentose is derived from this

cell following hæmolysis is considered in the discussion. It is concluded that, with the possible exception of the changes after scalding in the rat, the red cells are not the main source of the excess pentose. Some evidence in favour of the main source being the damaged tissue is presented.

The relationship of these results to other of our findings is discussed and it is concluded that trauma is accompanied by an increase in the concentration of nucleotide metabolites in the blood stream.

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SHORT ARTICLES

THE EFFECT OF LIMB ISCHÆMIA ON THE MAGNESIUM CONTENT OF PLASMA

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We have previously shown (Green and Stoner, 1944) that the administration of sub-anæsthetic doses of $MgSO_4$ to animals increases their sensitivity to various forms of tissue injury. The period of limb ischæmia required for a fatal issue is greatly reduced in the Mg-treated animal. Such an effect is obtained with all the usual laboratory methods for inducing shock, including the administration of adenosine triphosphate. If the degree of trauma is of itself lethal the effect of $MgSO_4$ is to cause a great decrease in the survival time. Such findings raised the possibility that Mg^{++} itself might be concerned in the production of the general bodily reaction to trauma. As a preliminary step in the elucidation of this relationship we have examined the total Mg level in the plasma of the rabbit after limb ischæmia.

Several workers (Lam, 1941 ; Moon *et al.*, 1941 ; Ricca *et al.*, 1945 ; Root *et al.*, 1947) have incidentally reported increases in the Mg level of the plasma in traumatic shock and burns. For the most part these authors have failed to take into account the influence of the anæsthetic used and of the depression of renal function which accompanies shock. It has been shown by Scholz *et al.* (1945) that nembutal produces an appreciable increase in plasma Mg and we have also found this to be the case with chloralose. It is well known that the plasma Mg increases in renal failure, but it would appear that the degree of failure has to be severe and of long standing before any appreciable rise occurs.

Methods

Six rabbits were anæsthetised with ether and 4·5 ml. of blood were removed by cardiac puncture. Tourniquets were then applied to both hind limbs for 5 hours by the method of Bywaters and Popják (1942). Two hours after the release of the tourniquets a further blood sample was withdrawn. None of the animals so treated survived 24 hours. Autopsy showed that death was not due to hæmopericardium and a series of similarly bled controls survived indefinitely. Heparin was used as anticoagulant and the plasma Mg was determined by the method of Hald (1933). The effect of renal failure was determined in a series of 5 rabbits in which the plasma Mg level was examined before and 7 hours after ligature of both ureters under ether anæsthesia.

Results

Examination of the blood 2 hours after a 5-hour period of limb ischæmia showed an increase, statistically significant at $P = 0.05$, in the Mg content of the plasma in all but one of the six animals tested (table I). The average

TABLE I

*The effect of 5 hours' bilateral limb ischæmia on the plasma Mg of the rabbit.
Blood specimens obtained by cardiac puncture*

Rabbit no.	Plasma Mg (mg. per 100 ml.)		
	Initial	2 hr. after release of tourniquets	Percentage change
47/4	2·34	4·00	+71
47/7	2·46	3·49	+42
47/8	3·32	4·53	+36
47/10	3·48	3·10	−11
47/16	1·65	2·70	+64
47/20	1·58	2·58	+63
Average percentage change . .			+44

TABLE II

*The effect of cardiac puncture and ether anæsthesia on the
plasma Mg level of the rabbit*

Rabbit no.	Plasma Mg (mg. per 100 ml.)		
	Initial specimen	Specimen 7 hr. later	Percentage change
47/5	3·36	2·44	−27
47/6	2·15	1·81	−16
47/9	3·80	3·54	−7
47/11	1·74	3·08	+77
47/17	2·36	2·70	+14
47/19	2·30	1·81	−21
Average percentage change . .			+5

TABLE III

*The effect of ligating both ureters of the rabbit under ether anæsthesia on the
plasma Mg level. Blood specimens obtained by cardiac puncture*

Rabbit no.	Plasma Mg (mg. per 100 ml.)		
	Before ligature of ureters	7 hr. after ligature of ureters	Percentage change
47/15	1·31	1·04	−21
47/21	1·68	1·73	+3
47/22	1·42	1·76	+24
47/24	3·14	3·81	+21
47/25	3·30	4·58	+39
Average percentage change . .			+13

increase was 44 per cent. The changes in the control group (table II) were not significant. In several of the control rabbits the plasma Mg level tended to fall slightly in accordance with the view of Brookfield (1933) that simple hæmorrhage is accompanied by a decrease in the plasma Mg content due to hæmodilution. Complete suppression of urine for the period of the experiment (table III) did not cause any significant change in the Mg level.

Discussion

These results show that limb ischæmia is followed by an increase in the total Mg content of the plasma which cannot be attributed to the method of sampling or the type of anæsthesia. Furthermore this increase cannot be explained by the depression of renal function *per se*, although this may play a part by preventing the excretion of excess Mg liberated into the blood stream. The most likely source of this excess Mg is the damaged tissue itself.

These results seem to supply some evidence for the hypothesis that trauma is associated with a disturbance in the distribution of Mg within the body. The possible importance of this has been indicated in the introduction and the evidence presented here would be greatly strengthened if it could be shown that the toxicity of adenosine triphosphate was greater in the animal after limb ischæmia than in the normal animal. As yet we have not been able to show this. Further work is necessary to determine the time relations of the increase and also to discover whether the increase affects mainly the ionised or non-ionised fractions of the total Mg content which has been estimated in these experiments.

Summary

Limb ischæmia in rabbits is followed by a significant rise in the total magnesium content of the plasma.

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616 . 5 . 001 . 17 : 612 . 129 (adenosine)

THE EFFECT OF FATAL CUTANEOUS BURNS ON THE ADENOSINE EQUIVALENT OF THE BLOOD OF RABBITS

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In previous experiments (Stoner and Green, 1944, 1945) we have shown that when the rabbit is exposed to various forms of trauma the adenosine equivalent of the blood increases. Cullumbine (1947) has recently found that mild scalding of the skin of the rabbit is accompanied by a slight but significant increase (27 per cent.) in the adenosine equivalent of the blood. In the present paper we show the changes which occur in this fraction of the blood after a rapidly fatal scald and compare them with the changes which occur after the injection of a dose of adenosine triphosphate (ATP) which kills the rabbit in a similar time.

Methods

The fur of the 6 rabbits used in this investigation was clipped short up to the level of the costal margin. A control specimen of blood was removed by cardiac puncture and the animal was then anæsthetised with Nembutal given intravenously and kept anæsthetised until death. While anæsthetised, the animals were immersed in water at 70° C. up to the level of the costal margin for 1 min. They were then removed and dried. Further specimens of blood were taken at intervals after scalding. A similar series of 6 control rabbits was treated in the same way except that they were not scalded. The whole-blood samples (2·0 ml.) were extracted by the method of Barsoum and Gaddum (1935) and assayed against adenosine on the guinea-pig atrium preparation of Drury et al. (1937-38) as modified by us (Stoner and Green, 1944). Each extract was assayed on two separate preparations and the figures given are the averages of the values obtained, corrected for variations in the hæmoglobin concentration of the blood by bringing them all to the level of 100 per cent. on the Haldane scale.

TABLE I

The effect of scalding on the adenosine equivalent (µg./ml. of whole blood corrected for Hb. percentage)

Rabbit no.	Before scalding	Time (hr.) after scalding when specimen taken					
		½	1	1½	3	4	5
58	200	330	300	300	...	290	...
61	220	240	290	...	260	240	...
62	240	270	310	...	230	300	220
63	190	220	200	200	230	...	...
69	110	170	110	220	...	...	...
70	210	280	300	...	340	...	...

ATP injections were given intramuscularly in the form of the Mg salt prepared from BaATP (Boots). The level of the adenosine equivalent of the whole blood was examined before and at intervals after the injection by the methods described.

Results

All the scalded animals died within 1½-5 hr. after scalding, the average time being 3·4 hr. The effect of this trauma on the adenosine equivalent of the

whole blood is shown in table I and these values should be compared with those in the series of control rabbits (table II). These results show that whereas

TABLE II

The effect of repeated blood sampling by cardiac puncture on the adenosine equivalent ($\mu g./ml.$ of whole blood corrected for Hb. percentage)

Control rabbit no	Time (hr.) when specimen taken							
	0	½	1	1½	2	3	4	5
65	230	160	160	...	...	130	180	200
66	230	220	200	...	...	200	180	220
67	190	190	190	...	170	170	190	...
68	210	220	180	...	180	210	130	...
73	290	270	300	310	...	290	300	...
74	330	330	320	290	...	290	280	...

the adenosine equivalent in the control animals tended to fall somewhat over the experimental period, the scalded animals showed a significant increase ($P = 0.05$), which reached a peak between 1 and 3 hr. after the injury.
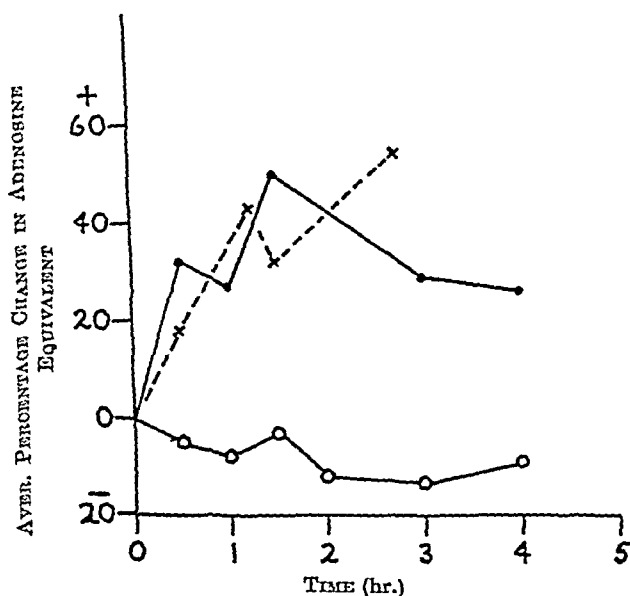
FIG.—Comparison of the average percentage changes in the adenosine equivalent of the blood after scalding, after the intramuscular injection of a rapidly lethal dose of ATP and after the withdrawal of samples of blood by cardiac puncture.

●——● After scalding at 70° C. for 1 min. at zero time.
x——x After the injection of 600 mg. MgATP per kg. body wt. at zero time.
o——o After repeated cardiac puncture and Nembutal anæsthesia.

It was found that the dose of ATP required to kill the animals in a similar period of time was 600 mg. MgATP per kg. body wt. I.M. With this dose the

animal died in a state of shock after 2¾ hr. The percentage changes which occurred in the adenosine equivalent after this dose of ATP are shown in the figure, where they are compared with the average percentage changes after scalding.

Discussion

These results indicate that severe thermal trauma is associated with an increase in the adenosine equivalent of the blood in the rabbit. The source of these increased amounts of adenyl compounds is probably the damaged tissue. Since whole blood was used in these experiments, the possible release of these compounds from the red cells in the hæmolysis associated with burns does not arise.

When these results are considered with those of Cullumbine there would appear to be a definite gradation of response according to the severity of the injury.

Under our experimental conditions death occurred at a similar time after both scalding and the injection of ATP, and the similarity of the changes in the adenosine equivalent of the blood in these two conditions is very striking. These results emphasise once again the resemblance between the state of shock produced by ATP and the naturally occurring condition.

Summary

Severe scalding causes a statistically significant increase in the adenosine equivalent of the blood of the rabbit.

The injection of a dose of adenosine triphosphate which kills the animal in a similar period of time gives a similar rise in the adenosine equivalent of the blood.

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582 . 28 (*Cryptococcus farciminosus*) : 576 . 8 . 093 . 31

THE YEAST-LIKE FORM OF CRYPTOCOCCUS FARCIMINOSUS (RIVOLTA): (HISTOPLASMA FARCIMINOSUM)

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(PLATE XXVII)

Cryptococcus farciminosus is the fungus responsible for epizootic lymphangitis, a disease of the Equidæ characterised by nodules and chronic suppurating ulcers on the skin, with inflammation of the associated lymphatic vessels and glands. In 1883, Rivolta and Micellone described in these lesions a parasite which they named *Cryptococcus farciminosus*. It was first isolated by Marcone in 1895, and in 1896 by Tokishige in Japan. Tokishige described the oval, thick-walled, budding yeast-like cells in pus, and the septate hyphæ and spores composing the growth on artificial media. Since then, cultures have been obtained on a number of occasions, the extensive work of Boquet and Nègre (1914-1919) deserving special mention, but only the mycelial form of the fungus has so far been grown.

This report describes methods of culture which permit growth of the yeast-like forms hitherto found only in animal tissues.

Isolation of the fungus

The cultures for the present study were isolated in 1945 and 1946 from cases of epizootic lymphangitis among mules used by the 14th Army in Burma. Pus from unruptured cutaneous nodules or lymphatic tissue from animals killed for post-mortem examination was sown on Sabouraud's agar or Hartley digest agar, pH 7·4, containing 10 per cent. horse blood. The tubes containing the media were sealed before incubation. At first duplicate sets of tubes were incubated, one set at 37° C., the other at room temperature; later, incubation was done only at 37° C.

Thirty-five attempts were made to isolate the organism from material in which the fungus was seen microscopically. Eleven cultures were obtained, representing strains from 5 cases. Seven of the 11 cultures were grown directly from the 5 infected animals and 4 from 3 mules and 1 donkey inoculated with pus from 3 of these cases.

The first culture was isolated on Sabouraud's agar after 12 days at 37° C., the fungus growing out of a clot of blood in the pus. No growth resulted at room temperature. Subsequent trial showed that the Hartley blood agar was superior to the Sabouraud agar. The remaining 10 cultures were isolated on Hartley's blood agar after periods of incubation at 37° C. ranging from 12 days to 8 weeks.

Primary colonies appeared as minute grey flakes in the layer of pus on the surface of the slope. Growth was slow. The colonies became heaped up, and wrinkled into a series of ridges and folds. They were adherent to the medium and difficult to emulsify. Microscopically the growth was seen to consist of a septate mycelium and chlamydospores.

Morphology of C. farciminosus *in infected tissues*

In the tissues of an infected animal the fungus appeared as an oval, budding, yeast-like organism, 2·5-3·5 μ long and 2-3 μ wide (fig. 1). Usually, one pole

was a little more sharply pointed than the other, giving a lemon-shaped outline. A clear unstained space around the majority suggested the existence of a capsule. Situated within large mononuclear or giant cells, the organisms were arranged singly or in groups, and were often so numerous that but little cytoplasm of the parasitised cell remained.

The cell wall of the fungus was very thick and highly refractile and did not stain. In fresh preparations, the cytoplasm appeared finely granular and usually contained one or more small hyaline bodies in active Brownian movement. When fixed and stained, many of the organisms showed large vacuoles, the cytoplasm being arranged around the periphery or concentrated into a crescent-shaped mass.

Budding was observed at the poles of cells, usually from the pointed end, sometimes from both. A narrow thread of cytoplasm connected the daughter and parent cells.

This parasitic yeast-like form of *C. farciminosus* closely resembles the parasitic stage of *Histoplasma capsulatum*, an observation first recorded by Da Rocha Lima in 1912.

The yeast-like form of C. farciminosus

Attempts were made to grow the yeast-like form of *C. farciminosus* in sealed tubes of a variety of media incubated at 37° C. A single strain was used for the first experiments, as it had a greater tendency than the others to produce a small number of yeast-like forms. Media tried included Hartley agar, pH 7·4, containing horse blood in concentrations ranging from 10 to 50 per cent., or with 10 per cent. sheep, ox, goat, pig, rabbit or dog blood; Francis's glucose-cystine rabbit-blood agar and a modification of this medium containing 10 per cent. horse blood; Salvin's peptone medium with 0·175 per cent. agar; and a modified Czapek-Dox medium with agar concentrations ranging from 0 to 0·5 per cent.

With all these, the growth was rough, wrinkled and adherent to the surface of solid media. Microscopically, it consisted of a pseudomycelium of short septate hyphæ with numerous thick-walled, round or oval chlamydospores, 5 to 10 μ in diameter. These were usually vacuolated and contained a number of large hyaline fat droplets. An occasional yeast-like form could be found, especially in old cultures.

The effect of carbon dioxide

When the culture was grown in a McIntosh and Fildes jar on Hartley blood agar at pH 7·4 in an atmosphere containing 15-30 per cent. CO_2, a slow but progressive change to the yeast-like form was observed. Subcultures were made every ten days. The first subculture was more easily emulsified than usual, about half the cells being in the yeast-like form. After the second and third subcultures the growth was smooth, pasty, moist and almost entirely yeast-like.

The yeast form was derived from terminal cells of the pseudomycelium, which became oval and swollen, and finally separated (fig. 2). Some were formed by direct budding from chlamydospores. These yeast-like cells were round or oval, 2-4 μ long, and similar in general structure to the parasitic stage, though not so uniform in size. Budding forms were very numerous, daughter cells usually developing from the more pointed pole, often from both, and occasionally from the sides. The culture also contained a few large round chlamydospores and short fragments of mycelium.

The optimum concentration of CO_2 appeared to be in the region of 15-20 per cent. A mixture of mycelial and yeast forms was found in cultures incubated in CO_2 concentrations of 10 per cent. or below, and growth was scanty in

CULTIVATION OF C FARCIMINOSUS

FIG 1 —Film of pus from a cutaneous ulcer on a mule showing yeast-like forms of *Cryptococcus farciminosus*. Gram. ×*ca*. 800.

FIG. 2 —*Cryptococcus farciminosus*. Yeast phase grown on blood agar at pH 7·4 in an atmosphere of 20 per cent. CO_2. Left : yeast like forms developing. Right fully-formed separated yeast forms. Gram. ×1600.

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concentrations of 30 per cent. or above. Without 15-30 per cent. CO_2, the culture could not be maintained in the yeast phase on Francis's glucose-cystine blood agar, Salvin's semi-solid medium or the modified Czapek-Dox medium. Cultures in sealed or unsealed tubes reverted in a few days to the mycelial type of growth, with the production of numerous large chlamydospores.

It was thought that a reduced oxygen tension in the McIntosh and Fildes jars might be responsible for the growth of the yeast form and experiments were made to test this idea. The yeast phase was sown on blood agar and incubated in jars containing 15 or 20 per cent. nitrogen instead of CO_2. Large quantities of soda lime (Indicarb) had been placed in the bottom of the jars to absorb the CO_2 remaining in the atmosphere and any formed during growth. Under these conditions the culture slowly reverted to the mycelial phase. The first subculture contained many yeast forms and a few chlamydospores and pseudomycelia. After four subcultures at intervals of 10 days the growth was rough, wrinkled and adherent to the surface of the medium, and consisted almost entirely of large round or oval chlamydospores with a few yeast forms and mycelial fragments. These results supported the idea that a suitable concentration of CO_2 was essential for the development and maintenance of the yeast-like form. So far 6 of the 10 cultures examined have been converted to the yeast-like phase by growing them on blood agar at 37° C. in 15 per cent. CO_2. The other 4 cultures produced abundant chlamydospores and hyphæ but only a few yeast forms.

The yeast phase could be re-converted to the mycelial phase on any suitable medium incubated at 22° C. After 18 hours the yeast forms produced short germ tubes and a septate branching mycelium bearing numerous chlamydospores rapidly developed.

The effect of CO_2 on other pathogenic fungi

It was found that the yeast-like phase of 3 strains of *Histoplasma capsulatum*, 2 of *Blastomyces dermatitidis* and 1 of *Sporotrichum schenckii* could be easily obtained on a simple peptone medium incubated at 37° C. in an atmosphere of 15 per cent. CO_2.

DISCUSSION

The presence of a certain concentration of CO_2 in the atmosphere appeared to be essential for the development of the yeast-like form of *C. farciminosus*. Carbon dioxide may also play an important part in the development of the yeast phase of other pathogenic fungi such as *H. capsulatum* or *B. dermatitidis*. Conant (1941) found that the yeast phase of a strain of *H. capsulatum* could be obtained in sealed but not in unsealed tubes of rabbit-blood agar. Salvin (1947) successfully grew the yeast phase of *H. capsulatum* in a simple semi-solid medium. Both sealed tubes and semi-solid media probably allow a local concentration of CO_2, and it is possible that *H. capsulatum* is more sensitive than *C. farciminosus* to CO_2.

Summary

1. The yeast-like phase of *Cryptococcus farciminosus* was obtained on blood agar incubated at 37° C. in an atmosphere of 15-30 per cent. CO_2.

2. The presence of CO_2 appears to play an essential part in the development of the yeast-like phase.

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$$576 . 8 . 093 . 33 : 576 . 851 . 49$$

SHALLOW-LAYER FLUID ENRICHMENT METHOD FOR INTESTINAL PATHOGENS

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During the recent war, supplies of sodium desoxycholate were so limited that it was necessary to modify the original media of Leifson (1935) by three substitutions: (1) sodium tauroglycocholate (British Drug Houses of B.P.C. standard) for the desoxycholate, (ii) peptically digested sheep serum for the pork infusion, and (iii) rosolic acid for the neutral red. These citrate-rosolic acid (C.R.A.) media (Brodie, 1942), both fluid and solid, were successfully employed in this laboratory for the isolation of dysentery bacilli from morbid material. The use of the fluid medium for enrichment purposes proved of advantage in the examination of stools from cases convalescent from dysentery (Jamieson *et al.*, 1944; Brodie *et al.*, 1946).

Further work, with both solid and fluid media (Brodie, 1948; Brodie and Shepherd, 1949), on the differential inhibition of coliform bacilli and rough variants of intestinal pathogens (Brodie, 1948) indicated that the smooth variant of a Sonne group III (Sonne, 1915) strain of *Shigella sonnei* was relatively tolerant of the high electrolyte content of the bile-salt media. In such media, the rough variant of the Sonne III strain and various coliform bacilli were inhibited on the surface of the solid media and in shallow broths, whereas the smooth variant of the Sonne III strain grew well.

The C.R.A. media were employed for direct plating and for enrichment by inoculating a 4-mm. loopful of fæces on to the plate and into 5 c.c. of the broth contained in a 1 oz. screw-capped bottle. The broth was plated on to the C.R.A. agar after 16-24 hr. at 37° C. In view of results of differential inhibition experiments, it was decided to employ also shallow enrichment broths of 0·5 c.c. of C.R.A. fluid medium contained in 5-c.c. screw-capped round bottles. This procedure gave a broth layer approximately 2 mm. in depth compared with the 17 mm. depth of 5 c.c. of broth in a 1 oz. bottle. For the shallow enrichment

broths, a 2-mm. loopful of faeces was used as the inoculum, and subsequent plating was done on C.R.A. agar as for the 17 mm. deep broths.

To date, 100 consecutive specimens of faeces have been examined in this way. In the absence of an outbreak of intestinal infection within the period of the test, the results reported are those from sporadic infections. From the 100 faeces so examined no positive isolations were made by direct plating or after enrichment in 5 c.c. deep broth, but 9 positive results followed enrichment in 0·5 c.c. shallow broth. Of these 9 isolations 4 were *Sh. sonnei*, 1 was *Sh. flexneri*, 2 were *Salm. aertrycke* and 2 were paracolon bacilli.

Summary

In the examination of 100 stools from sporadic cases of suspected intestinal infection with citrate-rosolic acid (C.R.A.) medium for intestinal pathogens, enrichment in shallow-broth proved more efficient than enrichment in deep broth, yielding nine positive isolations against none. This superior efficacy is likely to be most marked in mild infections and convalescent cases, *i.e.* where there are few infecting micro-organisms in the stools.

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TWO INDEPENDENT FOCI OF INTRADUCT CARCINOMA OF THE BREAST, ONE WITHIN A FIBRO-ADENOMA

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(PLATES XXVIII AND XXIX)

Malignant change in the epithelial element of a fibro-adenoma of the breast is curiously rare, although invasion of a fibro-adenoma by malignant cells from a nearby carcinoma has been described by Cheatle and Cutler (1931) and Willis (1948). Cheatle and Cutler state incidentally that carcinoma originating in a fibro-adenoma must be a very rare event. Their nearest approach to this was a small fibro-adenoma with epithelial malignancy apparently in continuity

with similar carcinomatous change in the ducts and acini of the adjacent breast. This tiny fibro-adenoma was discovered on microscopic examination and all that could be said was that the epithelium lining its clefts was identical in character with the intraduct malignancy elsewhere in the breast.

In a review by Harrington and Miller (1940), 15 cases of carcinoma of the breast are described as probably originating in the epithelium of a fibro-adenoma, 9 being alive 5 years after removal. The only tumour illustrated is said to be a grade I adenocarcinoma and from the illustration, a photomicrograph at ×26, it is impossible to say whether their term "adenocarcinoma grade I" means merely intraduct proliferation of benign type, or an early intraduct carcinoma. It is doubtful, therefore, whether the cases in this series are acceptable, not least in view of the marked contrast with authoritative opinion.

Four cases of carcinoma believed to have arisen within fibro-adenomata were discovered by Rose (1942-43) in a series of 302 cases of mammary carcinoma, and in the complex malignant mammary tumour described by Tudhope (1939) there were both sarcomatous and carcinomatous elements, the latter thought to have arisen in the epithelium of a fibro-adenoma.

The present case not only shows malignant change in the epithelium of a fibro-adenoma, but, despite the presence of another malignant focus within the same breast, there is also evidence to show that the changes in the fibro-adenoma arose there independently.

Case report

Clinical history. Mrs E. W., age 45, was admitted on 13.10.47 complaining that the right breast had "felt irritated" for the past five months and that there had been progressive in-drawing of the nipple. A freely mobile lump "the size of a pigeon's egg" was felt beneath the nipple, and a diagnosis was made, on clinical grounds, of carcinoma without involvement of axillary lymph nodes. Radical mastectomy, 15.10.47.

Pathology. A hard irregular mass lay immediately under the slightly retracted nipple; macroscopically it was a scirrhous carcinoma measuring approximately 5·0 × 3·0 cm. in diameter and about 3·5 cm. in depth. In the axillary tail, about 8·0 cm. from the main tumour, there was a hard round nodule measuring 1·5 cm. in diameter. On section, this seemed to be a lymph node replaced by metastatic growth. No other enlarged lymph nodes were found.

Microscopically the appearances of the main tumour are those of a scirrhous adenocarcinoma erupting from a still recognisable intraduct carcinoma; the infiltrating growth shows areas of obvious anaplasia, with numerous mitotic figures. In some of the mammary ducts close to the main tumour mass the lumen is still maintained and the epithelial appearances are those of the generalised malignant change which Muir (1941) has interpreted as being due to multiple foci of neoplastic change. Sections through the nipple show intraduct but no intra-epithelial or intra-epidermal spread.

The nodule from the tail of the breast proves microscopically to be a simple intracanalicular fibro-adenoma, sharply de-limited by a fibrous capsule and showing the usual epithelial-lined glandular spaces (fig. 1). This epithelium, however, has in many places the cytological characters of duct epithelium at an early stage of intraduct carcinoma. There is no sign of any neoplastic epithelium outside the ducts of the fibro-adenoma. Some of the clefts are lined by a simple, apparently normal, low cuboidal type of epithelium; others show piling up of the epithelium (fig. 2), enlargement of some of the cells, clear cytoplasm, nuclear hyperchromatism and a few aberrant mitoses. Some of the clefts are completely filled with carcinoma cells (fig. 3), frequently showing the so-called cribriform appearance (fig. 4). In general, the picture is identical with that of the intraduct carcinoma present elsewhere in this breast, and the
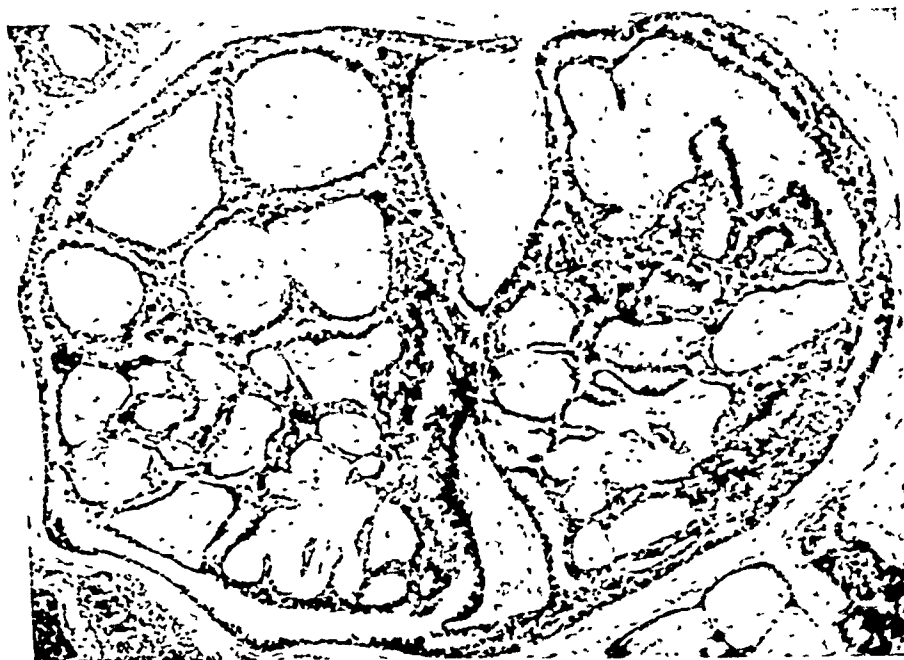
CARCINOMA ARISING IN A FIBRO-ADENOMA OF BREAST

FIG 1 —The fibro-adenoma , showing piling up of epithelium within the complex
extensions of the acini , this epithelium is cytologically identical with that seen
in the early stages of intraduct carcinoma, but there is no sign of eruption of
the neoplastic cells outside the glandular spaces. Celestin blue and hæmalum.
× 50.

FIG 2 —In the upper half of the field there is obvious filling and distension of the
glandular spaces by the proliferating epithelium, while in the lower half, the
clefts show, in places, merely a single layer of lining epithelium. The stroma is
very slightly cellular and in part hyalinised , there is no suggestion of sarcomatous
change. Celestin blue and hæmalum. × 65

CARCINOMA ARISING IN A FIBRO-ADENOMA OF BREAST
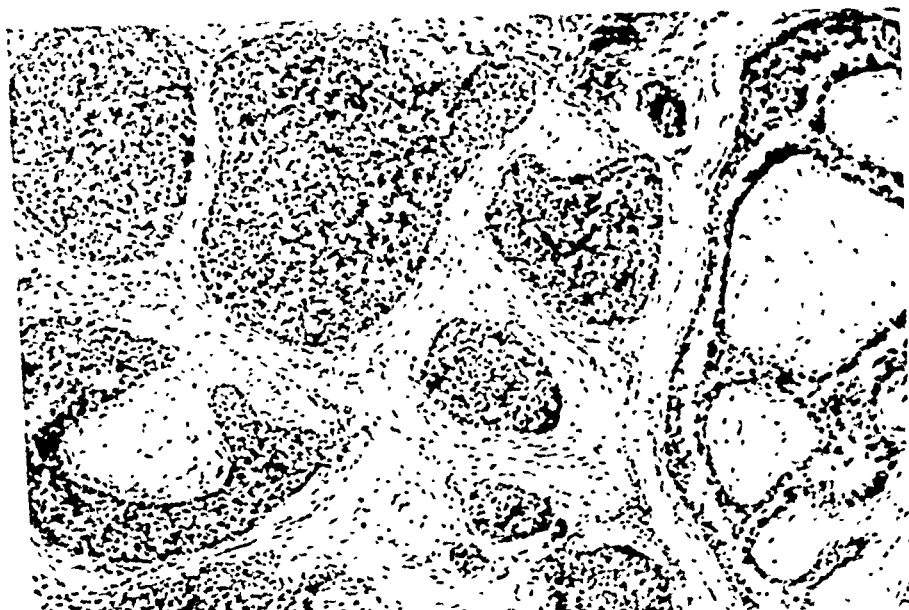
FIG. 3.—On the left of the field, the clefts are distended with cribriform alveolar carcinoma ; on the right there is a presumably earlier stage of the intra-acinar proliferation. Celestin blue and hæmalum.   × 105.
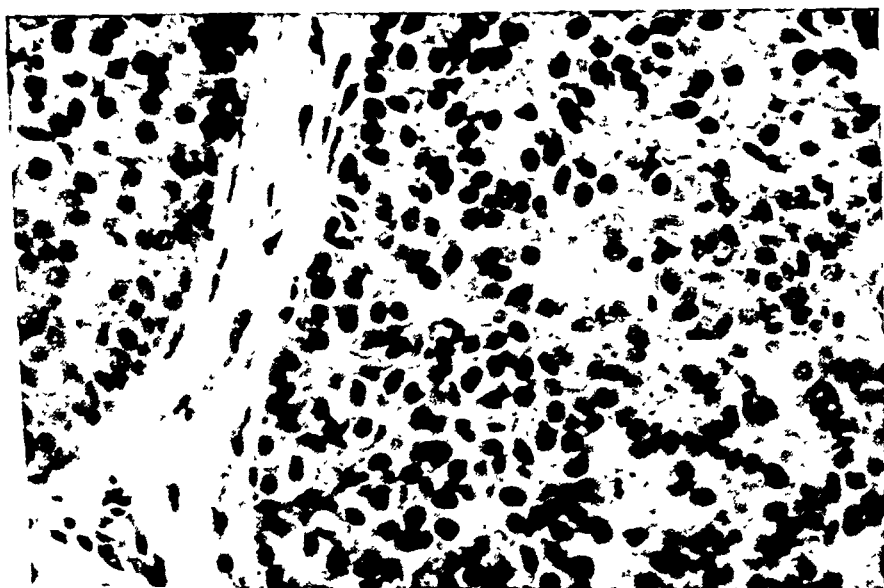
FIG. 4.—This higher-power view shows the tendency to glandular formation—the so-called cribriform arrangement. The epithelial cells are larger, paler and more varied in size than in the non-neoplastic zones, and there are a few mitotic figures. Celestin blue and hæmalum.   × 380.

question arises whether this is not in fact one of these breasts in which there is intraduct carcinoma throughout the ducts of the breast generally, as in Muir's case 9. The sites originally examined—the region of the scirrhous carcinoma and the fibro-adenoma, mistaken naked-eye for a lymph node—were of course chosen for obvious reasons. Fortunately the rest of the breast had been retained, and the zone between the two sites primarily examined was now subjected to detailed histological examination. This reveals that the ducts in the intervening area show no evidence of intraduct carcinoma and even close to the fibro-adenoma there is no sign of intraduct growth.

The possibility of secondary invasion of the adenoma by the carcinoma in the stroma of the breast was excluded with reasonable certainty by finding a zone of fully 4·5 cm. of tissue between the scirrhous growth and the fibro-adenoma in which no sign of malignant cells can be discovered.

Discussion

According to Cheatle and Cutler (1931) and Muir (1939), mammary carcinoma usually begins as an intraduct growth and the earliest changes frequently appear to be multicentric. From an interpretation of the histological appearances it seemed to them as if some widespread diffuse abnormality occurs in the duct epithelium, which is then followed by focal and frequently multiple plaques of proliferation ; the epithelium in turn gradually assumes the cytological characters of malignant epithelium and as such comes to fill up the available space in the ducts. Then at some point, for reason unknown, these obviously malignant cells traverse the basement membrane and there is carcinoma of the breast. The earlier stages of this process, beginning in diffuse hyperplasia and ending in recognisable intraduct malignancy, can all be seen in the epithelium of the present fibro-adenoma, and it seems reasonable enough to accept the possibility that the changes actually arose in the acini or clefts of the fibro-adenoma itself. It is known, of course, that the epithelium of an adenoma is responsive to endocrine stimulation, as in pregnancy and lactation (Geschickter and Copeland, 1945).

Thus in the present case there is an intraduct carcinoma of breast of fairly circumscribed type with an associated scirrhous carcinoma, and in the same breast a fibro-adenoma in the clefts of which there is also intraduct carcinoma. The intervening tissue shows normal ducts and it must be presumed that the two foci of intraduct growth are independent sites of origin. It is tempting to imagine that the epithelium in the clefts of the fibro-adenoma, being already abnormal, was thereby the more ready to react to the general stimulus which we may presume to have elicited the development of the intraduct carcinoma under the nipple. Such an idea is consistent with the suggestion put forward by Muir that endocrine disturbance may be the basis of the multifocal abnormalities in duct epithelium which go on to become intraduct carcinoma.

Summary

In a surgically removed breast there was discovered both an intraduct carcinoma with associated scirrhous carcinoma and, at a distance, a fibro-adenoma with intraduct carcinoma in its clefts. Examination of the intervening area fails to reveal any evidence of intraduct growth and thus the two zones of intraduct carcinoma appear to be independent.

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A SUGGESTED EXPLANATION OF THE ALLEGED PHENOMENON
OF SYMBIOTIC (BACTERIAL) NITROGEN FIXATION IN WHALES

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In 1933 Laurie postulated the existence of a symbiotic nitrogen-fixing micro-organism in large numbers in the blood of Blue and Fin whales (*Balænoptera musculus* and *B. physalus*). He referred to this as the X-organism, and suggested that it played a part in the dynamics of dissolved nitrogen during sudden pressure changes. This organism was tentatively endowed with the power of passing the placental barrier, though Laurie showed a proper caution in stating this.

Laurie's evidence for the existence of this organism rested upon microscopic examination and upon the disappearance of nitrogen in the dissolved phase from whales' blood taken at various periods after death; " in no case had the whale been dead more than twelve hours, and the majority less than six." Microscopic examination revealed small organisms of from 0·5 to 2·0 μ; their staining reactions are not reported.

There seems to have been some hesitation in accepting these findings, and few standard works of bacteriology refer to the X-organism, although the existence of such a symbiont might have considerable importance.

Scholander (1940) mentions that he was unable to demonstrate the X-organism in fresh whale blood, but saw " bacteria with whirling movements " in blood drawn from older carcases. No cultural or morphological studies are reported.

During 1946-47 I was engaged in a study of whale-meat bacteriology in the Antarctic and made some simple experiments to test Laurie's hypothesis. It had emerged, as reported elsewhere (Case, 1948), that the musculature of the whale becomes infected with a wide range of organisms very shortly after death, and that even after 1 hour the great muscles of the back may be appreciably contaminated. Blood was also found to be infected, though after a slightly longer interval, but some sterile specimens were obtained of both meat and

blood. The infection, a mixed one, consisted of many types of clostridia, including *Cl. chauvoei*, *Cl. œdematiens*, and some which are still unidentified, Gram-positive streptococci of fæcal type and Gram-negative rods. Studies of the bacterial flora of whale fæces suggested that the intestinal tract was the probable source of the contamination, but rigid proof of this is still lacking.

Methods and results

Experiments on whale blood were performed as follows. Six whales were sampled on different days. In two the blood proved to be sterile. Samples were taken under oil, with precautions against the introduction of infection. The nitrogen content was determined immediately and tubes of Robertson's cooked-meat medium were inoculated for a sterility test.

The remaining portions of the blood samples were then brought to equilibrium with atmospheric air in a revolving tonometer, two hours being allowed. This was carried out at 37° C. and aseptic precautions were maintained. The equilibrated blood samples were each divided into several portions, one of which was analysed and the rest placed in the incubator at 37° C.

Nitrogen analyses were carried out by the technique of Van Slyke and Neill (1924), and the results calculated to 760 mm. Hg.

The results obtained up to this point (table I) show that whereas the sterile blood samples were slightly supersaturated with nitrogen, which might be

TABLE I

Nitrogen-dissolving power and nitrogen content of whale blood at 37° C., the blood being equilibrated with air to estimate the dissolving power

Whale no	Type	Time post mortem (hours)	Sterility	N_2 (vols per 100 c c)	N_2-dissolving power (vols per 100 c c)	Percentage saturation
40	Blue	4	Sterile	3·78	3·32	113·9
41	Blue	1	,,	3 21	2·75	116·7
169	Fin	18	Infected	1·51	2·56	59·0
170	Blue	6	,,	1·32	2·44	54·1
174	Blue	5	,,	2·12	2·87	73·9
177	Blue	8	,,	1·95	2 65	73·6

expected in a recently surfaced animal, the infected samples showed a nitrogen deficiency comparable with that demonstrated by Laurie.

The equilibrated blood samples were incubated at 37° C. and portions analysed at 3-hourly intervals up to 9 hours. The results (table II) show that a nitrogen deficiency appeared in all the infected but not in the sterile specimens.

Other portions of the sterile blood samples were inoculated with a peptone-water culture of infected whale muscle taken twelve hours *post mortem* and incubated at 37° C., samples being taken for analysis as before. The results (table III) show that a nitrogen deficiency had appeared.

Portions of the infected blood samples were incubated after the addition of small amounts of penicillin (500 units per 10 ml.) and samples analysed as before. The results (table IV) show that nitrogen disappeared more slowly from these samples than from the same samples without penicillin (table II).

TABLE II

The disappearance of nitrogen from whale blood equilibrated with air at 37° C. and incubated at the same temperature

Blood no.	Sterility	Time from equilibration (hours)							
		0		3		6		9	
		N_2 (vols. per 100 c.c.)	Percentage* saturation	N_2 (vols. per 100 c.c.)	Percentage saturation	N_2 (vols. per 100 c.c.)	Percentage saturation	N_2 (vols. per 100 c.c.)	Percentage saturation
40	Sterile	3·32	100·0	3·35	100·0	3·30	99·4	3·31	99·7
41	,,	2·75	100·0	2·74	99·6	2·72	98·9	2·74	99·6
169	Infected	2·56	100·0	2·14	83·6	1·67	65·2	1·43	55·9
170	,,	2·44	100·0	2·03	83·2	1·59	65·2	1·18	48·4
174	,,	2·87	100·0	2·13	74·2	1·90	66·2	1·49	51·9
177	,,	2·65	100·0	2·01	75·9	1·67	63·0	1·38	52·1

* The blood in all these experiments was assumed to be 100 per cent. saturated after 2 hours in a revolving tonometer.

TABLE III

The disappearance of nitrogen from the blood of whales 40 and 41, infected with a culture from meat 39, equilibrated with air and incubated at 37° C.

Blood no.	Time from equilibration (hours)							
	0		3		6		9	
	N_2 (vols. per 100 c.c.)	Percentage saturation	N_2 (vols. per 100 c.c.)	Percentage saturation	N_2 (vols. per 100 c.c.)	Percentage saturation	N_2 (vols. per 100 c.c.)	Percentage saturation
40	3·32	100·0	2·41	72·6	1·76	53·0	1·51	45·5
41	2·75	100·0	2·10	76·4	1·79	65·1	1·41	51·3

TABLE IV

Nitrogen disappearance in infected blood equilibrated with air at 37° C. and incubated at the same temperature with 500 units of penicillin per 10 ml. of blood (cf. table II)

Blood no.	Time from equilibration (hours)							
	0		3		6		9	
	N_2 (vols. per 100 c.c.)	Percentage saturation	N_2 (vols. per 100 c.c.)	Percentage saturation	N_2 (vols. per 100 c.c.)	Percentage saturation	N_2 (vols. per 100 c.c.)	Percentage saturation
169	2·50	100·0	2·43	97·2	2·27	90·8	1·99	79·6
170	2·41	100·0	2·30	95·4	2·12	88·0	2·02	83·8
174	2·79	100·0	2·58	92·5	2·42	86·7	2·34	83·9
177	2·51	100·0	2·40	95·6	2·21	88·0	2·10	83·7

Discussion

Laurie's finding of nitrogen fixation in whales is confirmed, but it is possible that this effect is due to the extremely rapid contamination of the carcase which takes place within an hour or two of death and not to a symbiotic process. A foetus, in common with all other tissues, would be involved in the rapid spread.

Laurie found that the urine and allantoic fluid of otherwise " nitrogen-deficient " whales were supersaturated. This is probably because such fluids would not provide a suitable medium for nitrogen fixation, since nitrogen-fixing micro-organisms require a plentiful source of oxygen. In blood and muscle hæmoglobin and myoglobin would meet this demand.

There is nothing in this work to identify the nitrogen-fixer, but an extensive bacterial flora has been demonstrated in both blood and muscles analogous to the intestinal flora of the whale, which is presumably derived from the animal's food.

The occurrence in the sea of an abundant population of nitrogen-fixing bacteria of both Azotobacter and Clostridium types has been variously reported (Bavendamm, 1932 ; Benecke, 1933 ; Waksman *et al.*, 1933), so the concept outlined above seems well within the bounds of probability.

Summary

A bacterial nitrogen fixation was found to occur in whale's blood when the blood was infected as a post-mortem occurrence, but not in sterile blood.

It is concluded, therefore, that Laurie's nitrogen fixation is a post-mortem artefact and not evidence of symbiotic activity.

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MORPHOLOGICAL CHANGES IN BACTERIOPHAGE-INFECTED ORGANISMS AS REVEALED BY PHASE-CONTRAST ILLUMINATION

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(PLATE XXX)

Throughout the extensive literature on bacteriophage, references to the morphological changes which take place in infected bacteria are few and brief. Nothing of significance has been added to the account given by d'Herelle (1926)

who, using dark field illumination as his method of observation, described " inflated " and spherical forms which, after a variable period of observation, suddenly burst, leaving in the surrounding medium a slightly cloudy floccule which slowly dissolved. These swollen cells had a sharply outlined contour but no reference is made to internal structure. Wyckoff (1948), investigating the multiplication of bacteriophage by means of the electron microscope, suggests that young bacteria lyse soon after coming in contact with particles of bacteriophage and that multiplication of bacteriophage continues in the extruded protoplasm. This suggestion is difficult to reconcile with other established facts regarding bacteriophage, and it is probable that the technique of electron microscopy was responsible for the unexpected appearances which were observed.

During an investigation of the bacteriophages of *Salmonella typhi-murium* I studied the morphology of infected bacteria as revealed by phase-contrast illumination. The findings which were both definite and characteristic, are reported in this paper.

These bacteriophages may be divided into two main groups. Members of the first group always produce lysis in the organisms they infect. In artificial culture some types leave alive only a few resistant organisms, while others appear to achieve a state of balance in which sensitive bacteria and bacteriophage are found together. In neither case, however, do bacteria become permanently infected or lysogenic.

Members of the second group, after a preliminary destruction of sensitive organisms, produce an apparently harmless infection in the remaining cells which, under natural conditions, persists indefinitely. These infected organisms, therefore, become lysogenic.

Within these groups there are certain well-defined types which need not be detailed here. The descriptions which follow relate to three common bacterio-phages, of which the first destroys all but resistant organisms, the second achieves a state of balance without lysogenesis and the third destroys sensitive organisms but establishes permanent symbiosis with the remainder, producing a lysogenic culture.

Method of examination

Infected cultures in a broth medium are examined at the peak of growth preceding lysis. The bacteria are immobilised by imprisoning them in agar. A number of $3'' \times \frac{3}{4}''$ tubes are charged with about 0·5 c.c. of 2 per cent. nutrient agar which is kept melted by immersion in a water-bath at 45° C. An equal part of the culture to be examined is added to one of these tubes with a pipette and thoroughly mixed with the agar. A small drop of the mixture is then placed on a warm slide and pressed down under a cover slip to form a thin film. To prevent drying, the edges are sealed with melted vaseline. During examination the preparation is kept at 37° C. in a thermostatically regulated cell on the stage of the microscope. A powerful source of light, as for dark field illumination, is used and the beam is passed through a light-blue glass filter.

Findings

Normal bacteria. Uninfected normal bacteria in the logarithmic phase of development show active multiplication, and are short and oval in shape (fig. 1). They are of a uniform dark-blue, almost black, colour and have no obvious internal structure. Dividing organisms show an equatorial constriction which quickly deepens and halves the organism. In older cultures, which have been incubated overnight and left at room temperature for some time, the organisms are longer and dividing forms are scanty or absent, but otherwise they do not differ significantly from those in younger cultures. Occasionally, much elongated or swollen forms are seen. These are usually less dense than the normal bacteria,

but are equally structureless. They have been observed to remain unchanged, i.e. without undergoing lysis, over periods of 4-5 hours.

Infection with a bacteriophage which destroys all sensitive organisms (fig. 2). The earliest abnormality to be seen in an organism infected with this bacteriophage is the absence of division. As the body lengthens, the usual constriction fails to appear. Instead, a small mass of material which is lighter in colour than the normal bacterial cytoplasm develops centrally and grows until the bacillus is swollen to about twice its normal size. The dark bacterial cytoplasm is divided by this mass and pushed into the extremities of the cell where it can be seen as two polar bodies, the free edges of which are usually, though not invariably, concave and may be sharply defined. A few dark granules are frequently present in the light mass, particularly along its edges. Very occasionally, all the bacterial cytoplasm may be displaced to one extremity, leaving the rest of the bacterial body clear, except for a few isolated granules.

The use of the warm cell allows the stages of development leading to the lysis of the organism to be observed. While it is a comparatively easy matter to record the bursting of bacteria by making a diagram of a selected field and checking the disappearance of one organism after another by periodic counts, it may take half an hour or more of unremitting observation of a selected bacterium to see the actual burst. The uncertainty of the timing and the suddenness of the burst which, from beginning to end, takes less than 2 seconds, make it impracticable to record the changes photographically. The brush drawings shown in fig. 3 were made by Mr B. Jobling, and their accuracy is confirmed by the writer. The burst appears to result from internal pressure, which quite suddenly overcomes the resistance of the cell membrane. If previously curved or sinuous, the organism becomes straight and tense. A swelling develops either at one end or, more commonly, in the middle and the bacillus rapidly becomes blown up, though still appearing to have a definite limiting membrane. The burst then occurs and, after a transient haziness while the bacterial contents mix with the surrounding medium, only the remains of the dense polar masses can be seen. Usually these disappear within a few seconds but sometimes they persist as indefinite amorphous granules for some minutes. Occasionally, too, the " ghost " of the organism in the form of an empty cell membrane is momentarily visible.

Infection with a bacteriophage which achieves a state of balance (fig. 4). Under the influence of the second type of bacteriophage changes in the morphology of the organism differ from those seen in infection of the first type. In general, enlargement of the cell is greater. Many bacteria assume a club-like shape with an asymmetrical swelling near one extremity. The dense bacterial cytoplasm is again divided by the central mass of light material, and one portion is forced into the narrow end of the club while the other assumes *the form of* a band across the swollen end. Between the band and the adjacent pole there is a further clear area. In some, a small dark mass occupies the extreme tip of the bacillus at this end. Other bacilli show a long central constriction, and have light extremities and centre and two dark bands crossing the widest portion of each half. Occasionally, and particularly in the earlier stages, forms with dark bi-polar masses and clear centres resembling those produced by the first type of bacteriophage are also seen, but these are in a minority.

Infection with a bacteriophage which produces lysogenesis. When a culture of *Salm. typhi-murium* is exposed to the action of a potent bacteriophage of the second group, the immediate result is the infection and destruction of a considerable proportion of organisms which appear to have a higher degree of sensitivity than the others. In due course these susceptible organisms are eliminated and those remaining, although infected by bacteriophage, proceed to multiply normally.

The sensitive organisms, which are destroyed, undergo changes which

resemble, but differ in detail from, those seen in infection with the two previously described bacteriophages (fig. 5). They elongate to a greater extent but show little increase in width. The contrast between the denser and lighter areas is less marked and, while there are usually dark polar masses, these are relatively small. The body of the organism is crossed by irregular and ill-defined dark bands. In general, the light substance is more intimately intermingled with the dark than in the previous two.

The organisms which resist lysis and establish symbiotic union with the bacteriophage show no unusual characters and grow and divide in a normal way. An infected culture which has been maintained in the laboratory for two years was used to confirm this point and also to prove that the bacteriophage is present in each organism of an infected culture and is not merely kept alive through the agency of a few sensitive mutants. Portions of a young broth culture of this strain were simultaneously plated on agar and mounted for phase-contrast examination. The plate was placed in the incubator and the slide preparation in the warm cell for microscopic examination. A careful search of the latter failed to reveal any signs of bacteriophage infection or of lysis. One or two pale and swollen forms were seen but these underwent no further alteration. All other organisms were of normal appearance and divided repeatedly, so that in a few hours each had developed into a small massed group which was obviously the beginning of a colony. Next morning the incubated plate showed numerous well-scattered and discrete colonies, twelve of which were cultured and tested against a susceptible strain. Without exception, bacteriophage was found in each of them. The absence of any of the usual signs of bacteriophage infection in the microscopic preparation and the demonstrable presence of bacteriophage in all colonies from the plate provide strong evidence of the existence of a symbiosis in which there is only limited bacteriophage multiplication, so that the metabolism of the bacterium is not seriously upset by the presence of the bacteriophage.

Infection with other bacteriophages. Changes of a like nature, although differing in detail, were observed in the organism " coli B " infected with the T series of " coliphages " and also in infected typhoid and dysentery bacilli. In general, each bacteriophage produces in the bacterium a constant and, in some cases, a characteristic pattern composed of dark bacterial cytoplasm and the less dense substance which is absent from normal bacteria.

Discussion

The striking abnormality in infected organisms is the lighter substance which grows in and ultimately appears to disrupt the bacterium. There seems no reason to doubt that this is either the developing bacteriophage itself, or at least is closely associated with the formation of bacteriophage. This observation affords substantial confirmation of the generally accepted hypothesis that bacteriophage multiplies in and at the expense of the host bacterium. It does not support the theory that bacteriophage multiplication occurs in the bacterial proteins after they have escaped from the ruptured cell. Perhaps its chief interest, however, is that it indicates a method of investigation which may have a useful application in the study of viruses pathogenic to plants and animals.

Summary

1. Changes in the morphological characters of bacteria infected with bacteriophage can be seen by phase-contrast illumination.

2. These changes vary according to the bacteriophage but are constant for each bacteriophage and may be characteristic.

3. No changes are observed in a permanently infected lysogenic strain.

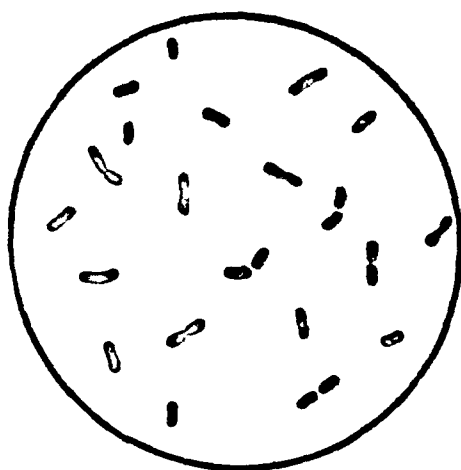BACTERIOPHAGE-INFECTED ORGANISMS

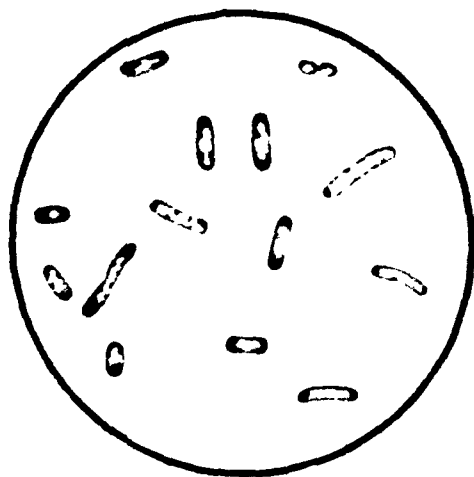FIG. 1.—Normal *Salm. typhi-murium* in the logarithmic phase of growth as seen by phase-contrast illumination. × 1500.

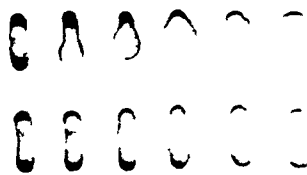FIG. 2.—*Salm. typhi-murium* infected with a non-lysogenic type of bacteriophage. × 1500.

FIG. 3.—Serial drawings depicting two types of "burst" in *Salm. typhi-murium* infected by a non-lysogenic bacteriophage. × 1500.

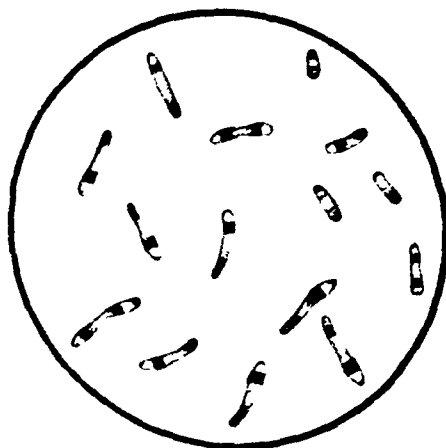
FIG. 4.—*Salm. typhi-murium* infected by another type of non-lysogenic bacteriophage. × 1500.
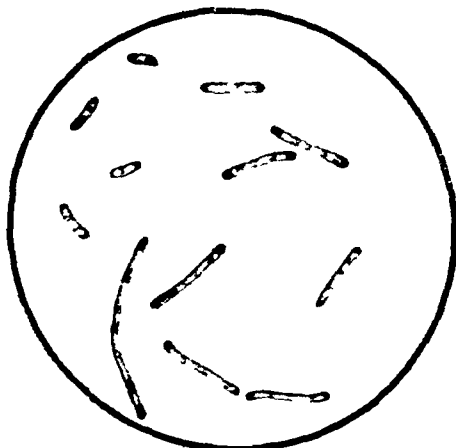
FIG. 5.—*Salm. typhi-murium* infected by a lysogenic bacteriophage. Bacteria showing these appearances are seen only in the early stages of infection of a culture. They ultimately burst. × 1500.

The figures are composite. The brush drawings, made to scale, depict a representative selection of the different forms observed.

4. This method of examination may have an important application in the study of plant and animal viruses.

I have to thank Mr B. Jobling for making the brush drawings which illustrate this note.

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WYCKOFF, R. W. G. . . . . . 1948. *Nature*, clxii, 649.

BOOKS RECEIVED

The dietary requirements of farm animals have been studied with some care and several textbooks have been produced which enable the stock owner to compile suitable rations. Comparable information on the diets of laboratory animals is far more extensive and has indeed grown to such formidable proportions that the reader who is not thoroughly familiar with the subject would need to search numerous journals to extract information on any specific point, as, for example, the influence of riboflavin on lactation.

In this monograph by Miss F. C. Russell a highly creditable effort has been made to bring together and to assess critically a mass of material : in this it serves a most useful purpose. The performance of a breeding colony may be influenced by the balance of the dietary constituents, *i.e.* the ratio of protein to carbohydrate, or by a deficiency or excess of an individual component. In order to bring more precision to the science of feeding, efforts have been made to elaborate diets from substances of known chemical composition. The author gives numerous examples of the care which must be exercised and shows how slight alterations may have a profound effect on the breeding female. In any colony the chief criteria for assessing the suitability of a diet for breeding purposes are (a) the percentage of females which become pregnant, (b) the size of the litter at birth, (c) the size of the litter at weaning, and (d) the effect of the diet on fertility and lactation.

There are many diets of natural foods which are recognised as giving satisfactory results and the first part of the monograph summarises the experience of previous workers with rations of this type. They are, however, subject to variations, and the results obtained in one institute may not be repeatable in another. Miss Russell rightly concludes that it is impossible to lay down a standard ration, but she gives sufficient information on the better-known stock diets to indicate the pitfalls.

The rest of the booklet covers the literature on individual components and is divided into sections on proteins, fats, carbohydrates, metals and various vitamins. The different species of laboratory animals show slight variations in their dietary needs. Naturally, most of the available information has been secured in the rat and mouse. There is a useful table on page 84 which summarises the success in rearing young attained by mothers fed on purified diets. It would be an advantage in future editions to enlarge this table to include other types of laboratory animals.

There is a comprehensive bibliography which is most valuable.

Although this book is not written for any particular class of reader, it is well worth the attention of any pathologist who is interested in the

133

quantitative chemical aspects of his subject. Micro-analysis is essential for both routine work and special investigations in chemical pathology, but many pathologists must be unaware of the wide range of micro-chemical techniques and methods now available to them. This book gives an up-to-date account of the subject, and the sections on volumetric analysis, colorimetry, electro-chemical methods and gasometric methods in particular may be strongly recommended to pathologists who wish to be informed of recent developments in these techniques. In each section, principles, procedures, sources of error and apparatus are competently dealt with, and are followed by many examples of analysis employing the techniques described. A considerable proportion of these examples deals with substances of biochemical or pathological importance, and while the collection of such examples is by no means exhaustive, it is nevertheless a very useful one.

The editors of this book are to be complimented on bringing together in one volume so much valuable instruction and information in the varied field of micro-analysis.

Mycoses and practical mycology

By N. GOHAR. 1948. London : Baillière, Tindall and Cox. Pp. xi and 234 ; 4 colour plates and 134 text figs. 25s.

Despite the recent publication of several useful books on various aspects of medical mycology, there remained a demand for a small book in simple language in which stress is laid on the mycoses rather than on mycology. Dr Gohar's little book, designed " for students and practitioners," is offered in response to this demand. A short introductory chapter on the characters of the fungi and the basis of classification is followed by a brief account of the mycoses, their epidemiology and parasitology. The remainder of the book is divided into chapters dealing with individual diseases or disease groups, and the final chapter contains a list of common fungicides, some prescriptions used in dermatological practice, the formulæ of several useful culture media and a note on poisonous mushrooms.

The book is well got up and beautifully illustrated by 134 photographs and line drawings, many of which are original. The complete omission of references to the relevant literature detracts greatly from the usefulness of the book, and more attention might have been paid to recent notable advances in medical mycology. In the matter of taxonomy and nomenclature the simplified system in common use to-day would have been preferable to the multitudinous specific names favoured by Dodge.

The physiology of domestic animals

By H. H. DUKES. 6th ed., 1947. Ithaca, N.Y. : Comstock Publishing Co., Inc. (English agents Baillière, Tindall and Cox). Pp. xii and 817 ; 1 colour plate and 183 text figs. 41s.

It must always be difficult to write a textbook on a subject that covers a wide field like the general physiology of domestic animals ; to decide what to include and what to omit ; to assign degrees of importance. That is true, on the assumption that the book is planned with a certain class of reader or a certain purpose in view ; and most subjects are now too big to make the preparation of compendia of knowledge either possible or desirable. We are told in the introduction that this book is designed for students of veterinary medicine and " will be useful to " students of animal husbandry. It may serve the examination needs of these students ; it

is less certain whether it will be of much use to them in practice. It is a curious mixture. In parts the writing is pretentious and obscure; in others there is over-simplification, almost to absurdity; for instance, the graph on p. 37 portraying the " correlation between erythrocyte count and hæmoglobin content of blood (dogs) " or the accompanying paragraph on hæmoglobinometers and conversion of hæmoglobinometer readings to grammes hæmoglobin. That does not mean that there are not excellent parts as well. The exposition of the meaning of pH is outstandingly simple and intelligible.

The general impression, to one not familiar with earlier editions, is of a compilation on classical lines which has been spoilt by being " brought up to date." The best parts appear to be either intact or rewritten; the worst are interlarded with bits and pieces like cards from a filing cabinet, inserted but not incorporated.

This bringing up to date of textbooks is a dangerous occupation. It is practised far too often in this form, and results in lack of continuity of thought, absence of co-ordination of presentation and, in its worst manifestation, the presence in the text of isolated statements, " So-and-so has studied such-and-such", which contribute nothing to the discussion and, if they must be included, should be in a supplementary bibliography of recent work. Such doctoring of a book destroys it. Surely the prime purpose of a textbook is to present a co-ordinated argument and reliable conclusions. A textbook is for the student, to provide a background. Research moves so quickly that no textbook can do more. The material for it should be selected with that in view; that and the purposes for which it is intended. If it is to be " brought up to date," that process should be limited to correcting errors in the original data, *e.g.* the (1916) data for hæmoglobin in human blood (p. 36) and the renal threshold for glucose in man (p. 502). (There are many other inaccuracies, surviving or freshly introduced.) Expansion should be by addition of new chapters. For example, the process of insertion here has scattered statements on anæmia and made the treatment confused; there is no systematic definition of terms or of stages in blood formation, and no orderly account of the nutritional anæmias. Some of the insertions are misleading or simply not informative.

It seems possible that a student of nutrition cannot view the contents of this book without prejudice. With due allowance for prejudice, it still appears necessary to say that for students of veterinary medicine and for animal husbandmen the treatment of nutrition seems inadequate. Even the best known nutritional deficiency diseases are briefly—even inaccurately—described, or dismissed in a sentence, however interesting the story. It is more surprising, and equally important, that there is no mention at all of immunology. But surely the physiology of resistance to disease should have a place in a textbook for veterinary students, even if it were only in respect of antibodies in colostrum and transmission across the placenta. To deal with such questions might incidentally arouse a lively interest in the complex physico-chemical phenomena sketched in the first chapter.

PROCEEDINGS OF THE PATHOLOGICAL SOCIETY OF GREAT BRITAIN AND IRELAND

31st December 1948 and 1st January 1949

The seventy-seventh Meeting of the Society was held, in two sections, at the London School of Hygiene and Tropical Medicine, and at University College Hospital Medical School, London, on Friday, 31st December 1948, and Saturday, 1st January 1949.

Communications and demonstrations

Those marked with an asterisk are abstracted below

*R. E. O. Williams and Ann Hirch. Streptococci in the air of occupied schoolrooms.

R. Cruickshank, H. J. Harris and A. J. H. Tomlinson. The role of the nasal carrier in outbreaks of streptococcal infection.

*J. Ungar. Agglutination of red cells by *H. pertussis*.

C. F. Barwell, I. M. Dawson and A. S. McFarlane. Electron microscopy of psittacosis virus.

J. W. Howie. Diet and resistance to infection.

*Jean L. Young, R. D. Stuart and W. Wilson. Control of a contaminant by adjustment of environment.

Joan Taylor and B. W. Powell. A serological type of *Bact. coli* associated with neo-natal and infantile diarrhœa.

K. Zinnemann. Sensitivity of *Hæmophilus influenzæ* strains to various sulphonamides.

*O. C. Lloyd and T. Sommerville. The fate of sporozoites of *Plasmodium cynomolgi* injected into the skin of *rhesus* monkeys.

Margaret Tutt and Janet Vaughan. Some observations on strontium metabolism in rabbits.

B. Lennox. Formation of melanin in epithelial tumours of the skin.

M. Bodian. The pathology of Hirschsprung's disease.

S. Sevitt. The physio-pathology of experimental skin burns.

F. W. Gunz. Some experiments *in vitro* with human leukæmic blood cells.

A. H. Cruickshank and T. P. B. Payne. Studies of the resistance to infection of rabbits with alloxan diabetes.

I. Doniach. Changes in the meningeal vessels in acute and chronic (streptomycin-treated) tuberculous meningitis.

A. C. P. Campbell. The pathogenesis of acute pancreatitis.

J. F. Heggie. The probable continuous nature of glomerular activity in the mammalian kidney.

E. Boyland and E. S. Horning. The induction of tumours with nitrogen mustards.

L. Dmochowski. Survival of the milk factor in transplantable breast tumours of mice.

L. Dmochowski and L. H. Stickland. Dialysation of the milk factor.

R. J. Ludford, J. Smiles and F. V. Welch.   Distribution of nucleotides during mitosis in malignant cells.

R. D. Passey, L. Dmochowski, W. T. Astbury and R. Reed.   Biological observations in electron microscope studies of high-breast-cancer strain tissues.

C. V. Harrison.   Primary pulmonary hypertension.

E. J. King, B. M. Wright and S. C. Ray.   Attempts to prevent experimental silicosis with aluminium.

A. G. E. Pearse.   The nature of Russell bodies.

J. S. Young, C. E. Lumsden and A. L. Stalker.   The estimation of the " tissue pressure " of normal and neoplastic tissues in the rabbit.   (Cinematograph film.)

J. W. Whittick.   Isolated amyloidosis of the trachea and bronchi.

R. E. Rewell.   (1) Carcinoma of the ovary in a goose.   *(2) Squamo-sebaceous carcinomas in the skin of a polecat (*Putorius putorius*).   *(3) Sweat gland tumour in a cotton rat (*Sigmodon hispidum*).   (4) Lymphatic leukæmia in an African brush-tailed porcupine (*Atherurus africanus*).

R. Rodda.   A case of *spina bifida* with meningo-myelocele and the Arnold-Chiari malformation producing hydrocephalus.

J. F. Heggie and Ivor Lewis.   (1) Benign calcified epithelioma of skin.   (2) Œsophageal carcinomata.   (3) Teratoma of anterior mediastinum.

J. F. Heggie and I. M. Tuck.   Twin placenta : hydrops fœtalis in one of Rh-positive binovular twins.

B. A. D. Stocker.   Experiments on di-phasic variation in salmonellæ.

A. Young.   An easy method of constructing perspex museum jars.

R. M. Fry.   Influence of medium on hæmolytic streptococci grown on blood-agar plates.

Abstracts

$$614 \cdot 71 - 095 \cdot 37 : 576 : 851 \cdot 21$$

STREPTOCOCCI IN THE AIR OF OCCUPIED SCHOOLROOMS

R. E. O. Williams and Ann Hirch

Central Public Health Laboratory, Colindale, London

The use of mouth streptococci to indicate respiratory pollution of air was apparently first suggested by Gordon (1904) ; but although Gordon's experimental observations have frequently been confirmed, none of the advocates of the test has attempted to demonstrate any correlation between the streptococcal count of the air of occupied places and the sickness prevalence among the occupants.   A trial of air purification in elementary schools offered us an opportunity for testing this correlation, and we accordingly developed a technique for the recognition of airborne streptococci.   This technique is the subject of the present note.

Using a slit-sampler modified from that described by Bourdillon, Lidwell and Thomas (1941), we collected the airborne bacteria on to a medium consisting of nutrient agar with 5 per cent. horse serum, 5 per cent. sucrose, 0·00025 per cent. crystal violet and 0·001 per cent. potassium tellurite.   The plates were incubated for 40 hours at 37° C.   So far as could be determined, this medium permitted the growth of the great majority of streptococci collected on it, and inhibited 85 per cent. of the total aerobic flora of the air, including all staphy-

lococci, most micrococci, and many spore-bearing and coliform bacilli and moulds. In the presence of the sucrose, the levan-producing streptococci referred to by Sherman, Niven and Smiley (1943) as *Str. salivarius* formed mucoid colonies 2-4 mm. in diameter, by which they could readily be recognised. Of the other colonies that developed on the medium, only about 10 per cent. were streptococci. and the exact proportion was determined in each sample by picking a random sample of the colonies into drops of nigrosin for microscopic examination.

With this routine we examined the relation of the total streptococcal count, estimated from the microscopic morphology of a sample of the colonies, and of the *Str. salivarius* count, to the amount of talking and physical activity among the children in the rooms, graded on an arbitrary scale. The relationship appeared to be linear, and the standard partial regression coefficients on talking and activity respectively in a series of 273 observations were : total streptococci +0·328 and +0·070 ; *Str. salivarius* +0·374 and +0·070 ; and in the same rooms the regressions of the total aerobic count on serum agar were +0·154 and +0·205. The regressions of the streptococcal counts on talking and of the total count on activity were all highly significant.

By further examination of 2095 colonies on plates from 30 rooms, the organisms growing on the selective medium could be classified as follows :

(a) Alpha- or non-hæmolytic streptococci that failed to grow on blood- or serum-agar containing 40 per cent. of bile formed 8·02 per cent. of the colonies (0·83 per c. ft. air). Of these, 29 per cent. (2·33 per cent. of all colonies ; 0·24 per c. ft.) were levan-producing *Str. salivarius* ; the remainder could be classed as *viridans* streptococci.

(b) Typical β-hæmolytic streptococci made up 0·1 per cent. (0·01 per c. ft.). Groups (a) and (b) appear to be largely mouth types of streptococci.

(c) Alpha-, β- or non-hæmolytic streptococci that flourished on serum agar containing 40 per cent. of bile made up 1·73 per cent. (0·18 per c. ft.). Of 58 tested, 39 reduced methylene blue in milk, and 8 of the 16 so far tested reacted with a Lancefield group-D serum. This group clearly contains a number of fæcal types of streptococci.

(d) Practically all the remaining 90·15 per cent. (9·35 per c. ft.) were pleo-morphic Gram-positive cocci, arranged in pairs or small clusters but never in chains, growing in semi-translucent colonies 0·5-1·0 mm. in diameter and producing a zone of greenish discolouration on blood agar. Their catalase-production was very weak and practically all the strains grew on 40 per cent. bile agar. We regard them as micrococci, though they almost certainly correspond to the " putative streptococci " of Buchbinder, Solowey and Solotorovsky (1938).

In our experience it is very unusual to find bile-tolerant streptococci or α-hæmolytic micrococci in the mouth and throat, and all such strains should therefore be excluded from an index of respiratory pollution of air. This can conveniently be done by subculture of the colonies from the selective medium to a blood-agar plate poured with a ditch of serum agar containing 40 per cent. of bile. In our samples practically all the strains that failed to grow on the bile agar proved to be *viridans* or *salivarius* types of streptococci. For a routine method, however, this is very wasteful of time, and it seems preferable to use the number of levan-producing *Str. salivarius* in an air sample as the indicator of respiratory pollution rather than the number of total or α-hæmolytic streptococci. This has the advantage that *Str. salivarius* can be recognised by colonial morphology alone without any possibility of confusion with fæcal streptococci or with the α-hæmolytic micrococci that greatly outnumber streptococci in the air of occupied places. It has the disadvantage, however, that owing to the small number of *Str. salivarius* usually present, large volumes of air, e.g. 20-100 c. ft., have to be sampled.

R. J. Ludford, J. Smiles and F. V. Welch. Distribution of nucleotides during mitosis in malignant cells.

R. D. Passey, L. Dmochowski, W. T. Astbury and R. Reed. Biological observations in electron microscope studies of high-breast-cancer strain tissues.

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added to each tube and, after careful shaking to ensure thorough mixing, the tubes were incubated in a water-bath for 2 hours.

The results were read immediately after incubation, the tubes being tapped and shaken until the deposited cells became re-suspended in the clear supernatant fluid. Reading was facilitated by using an oblique unilateral source of strong light with a dark background.

Our experiments showed that all the 32 virulent strains of *H. pertussis* tested agglutinated human red cells, irrespective of their blood group, up to a dilution of 1 in 8 to 1 in 16. Six avirulent strains of *H. pertussis* did not cause agglutination. Suspensions of cultures grown for 24 and 48 hours regularly agglutinated red cells. Cultures grown for 72 hours seemed to have lost their agglutinating ability to a considerable extent. We estimated the effect of different killing agents on the hæmagglutinating properties of the bacterial suspension. Bacteria exposed to 56° C. for half an hour showed only a slight drop in their agglutinating power. Bacteria treated with merthiolate (1 : 10,000) or tricresol (0·3 per cent.) seemed to have their agglutinating properties considerably reduced, and those treated with 0·5 per cent. formol or 0·5 per cent. phenol lost them entirely.

The agglutination of red cells takes place at temperatures between 42° and 50° C. At 52° C. some hæmolysis occurs after 1 hour. The anticoagulants heparin, citrate and oxalate seem to be equally suitable for this agglutination test. Blood defibrinated by shaking with glass beads can also be used, although the agglutination end-points are lower than with anticoagulants. The optimal concentration of red cells in the suspension for reading the agglutination result is about 5 per cent. We tested the red cells of mice, guinea-pigs, rats and rabbits and found that those of the rat reacted with more dilute *pertussis* suspensions than the others. Human red cells gave the next highest end-points. Red cells washed a few times with saline lose their agglutinability by *H. pertussis*. When the supernatant fluid from the first washing was added to the thrice-washed non-agglutinable red cells, they again became agglutinable. The water-soluble fraction of the red cells in the presence of which agglutination takes place is heat labile. Heating of the washings at 60° C. for one hour destroys the agglutinable substance.

My thanks are due to Mr P. W. Muggleton and Mr C. J. Hayter of these Laboratories for their technical help.

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576 . 8 . 093 . 31 : 576 . 8 . 095 . 16

CONTROL OF A CONTAMINANT BY ADJUSTMENT OF ENVIRONMENT

JEAN L. YOUNG, R. D. STUART and W. WILSON

From the Public Health Laboratory, Glasgow

Most laboratory workers have encountered serious culture-medium contamination due to one specific type of micro-organism. Generally the organism disappears after some trivial reorganisation of medium preparation and storage, but occasionally, as in the present case, its persistence becomes a major laboratory problem. The cultural characters of the contaminant placed it in the *subtilis*

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576 . 8 . 097 . 34 : 576 . 851 . 46 (*H. pertussis*)

AGGLUTINATION OF RED BLOOD CORPUSCLES BY *H. PERTUSSIS*

J. UNGAR

Research Division, Glaxo Laboratories Ltd., Greenford, Middlesex

In a previous report (Ungar and Muggleton, 1948) methods of differentiating between virulent and avirulent strains of *Hæmophilus pertussis* were described. These included, in addition to the mouse-virulence test and agglutination titre, alkali and bile solubility and precipitability with aluminium phosphate. The mechanism of these phenomena is not clearly understood, but there are indications that it is based on the difference in chemical composition between virulent and avirulent strains. Growth requirements of virulent and avirulent strains on semi-synthetic media (James, 1949) differ considerably and this certainly has a bearing on the structure of the bacterial cell. The report of the Australian workers (Keogh *et al.*, 1947) on the ability of virulent *pertussis* strains to agglutinate red blood cells seemed to us an additional important method of differentiation between virulent and avirulent strains, and worth further investigation.

We tried suspensions of freshly isolated *H. pertussis* strains, grown for 24 and 48 hours, for their action on human red cells. Bacteria suspended in saline in small test-tubes at 37° C. and kept for 1-2 hours did not, however, show any red-cell agglutination. We tried red cells in heparinised blood from different blood groups, supplied by courtesy of Drs Allott and Hillman of Lewisham L.C.C. Hospital, but without success. It seemed, therefore, that we could not obtain the results claimed by the Australian workers.

At this juncture it occurred to us to place the tubes containing red cells and bacterial suspensions in a water-bath at 46° C. Agglutination then ensued within 2 hours, irrespective of the type of cells used, being therefore one of the thermophile reactions which take place at higher temperatures. Having established the basic conditions for the occurrence of agglutination, we next investigated the factors concerned in the agglutination, with the following results.

Strains were grown on Bordet-Gengou medium unless otherwise stated. The organisms were harvested from the slopes with saline, the suspension centrifuged, the clear supernatant discarded and the organisms re-suspended in saline to give a concentration equivalent to about 6000 million organisms per ml. Serial dilutions from 1 in 1 to 1 in 16 of the suspension in 0·5 ml. quantities were prepared in Lambeth tubes of constant size and bore.

Heparinised blood (heparin 1 : 100,000) was centrifuged, the supernatant plasma discarded and the packed cells re-suspended in saline by gentle shaking. This was again centrifuged for about 5 minutes at moderate speed and the supernatant washings removed. The packed cells were again re-suspended in fresh saline to give a 5 per cent. concentration, 0·1 ml. of the suspension was

added to each tube and, after careful shaking to ensure thorough mixing, the tubes were incubated in a water-bath for 2 hours.

The results were read immediately after incubation, the tubes being tapped and shaken until the deposited cells became re-suspended in the clear supernatant fluid. Reading was facilitated by using an oblique unilateral source of strong light with a dark background.

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From the Public Health Laboratory, Glasgow

Most laboratory workers have encountered serious culture-medium contamination due to one specific type of micro-organism. Generally the organism disappears after some trivial reorganisation of medium preparation and storage, but occasionally, as in the present case, its persistence becomes a major laboratory problem. The cultural characters of the contaminant placed it in the *subtilis*

group and seemed nearest to those of *Bacillus cereus* (Breed, Murray and Hitchens, 1948). On plates used for the cultivation of gonococci it appeared in 12-24 hours as a flat moist rhizoid colony which rapidly spread as a confluent film over the entire plate. It was never noticed on other media. Each swab for gonococci was cultured on two blood-agar plates (Moffett, Young and Stuart, 1948), which were made slightly softer than usual. Cultures were incubated in biscuit tins with tightly fitting lids, and CO_2 was provided from marble chips dropped into 20 per cent. HCl kept in containers within the tins. The soft agar and the moisture present in the cultural environment were both found to be directly contributory to the worst feature of the contaminant—its spreading growth. The organism was not present in the specimens and apparently gained entrance during the process of plate inoculation, but it rapidly became so ubiquitous that it threatened the continuation of this branch of diagnostic work. The following steps were taken in an endeavour to eliminate it.

Disinfection of local environment. The organism was present in culture tins and incubators, on benches and in wall cupboards. Tins were autoclaved regularly and incubators and so forth were scrubbed repeatedly with 5 per cent. lysol. Afterwards the organism could not be cultured from the various surfaces, yet the incidence of plate contamination was practically uninfluenced.

Investigation and attempted control of source. The nature of the organism suggested that the animal house, which was within the laboratory building and on the same floor, and particularly hay fodder, might be possible sources. Attempts to isolate it from this area failed. The organism seemed to be present only in the room used for gonococcal work. Its recovery from the white coats and dusters used by workers in this room led to the daily sterilisation of these articles. Sterile hoods and rubber gloves were worn. Again, the organism was discovered in the face powder of one of the lady workers in the affected room. This source was eliminated. None of these procedures had more than a dubious and temporary effect in reducing the contamination. All internal sources of the contaminant seemed to be eliminated except, of course, its own remarkable multiplication whenever it established itself on culture media. External air contamination was feasible, but could not be confirmed, nor could it be eliminated, as the windows had metal frames and were not airtight. Accordingly, all gonococcal cultures were prepared inside a glass and metal bench cabinet which was irradiated with ultra-violet before and after use. In addition cultures were as far as possible completed before culture plates were examined. Only a slight temporary improvement was obtained. At this stage, with the use of gowns, gloves, hoods, and protected and disinfected environments, the contaminant was only reduced but not conquered and the original source remained undiscovered.

Adjustment of environment. The only course left lay in alteration of the immediate cultural environment. The organism required moist conditions to initiate its spreading growth. On gonococcal culture plates in the open incubator it grew as a discrete colony which did not spread. Successful gonococcal cultures required a moist environment and incubation in closed containers to maintain the concentration of CO_2, but attempts were made to reduce the progressive increase of moisture in this environment during incubation by removing one obvious source. The acid container was taken out of the tins after all CO_2 had evolved, or gas was supplied directly from a Kipp's apparatus. While all the precautions on sterility were maintained, a number of culture plates were incubated in parallel and on alternate days under these conditions (table I).

Both new measures produced a slight drop in the incidence of the contaminant but the result was still unsatisfactory. At this point one of us (W. W.) suggested the applicability of commercial moisture-control methods whereby constant humidities are obtained in closed compartments by water interchange with

saturated solutions of various salts. Lithium chloride was selected because it did not absorb CO_2 and maintained a low humidity—15 per cent. at 20° C. according to the Merck Index (1940). A saturated solution in a small beaker

TABLE I

Effect of environment on incidence of contaminants

Environment	No. of plates	Contaminants	
		No	Percentage
CO_2 from acid	94	9	9·6
CO_2 from acid; acid container removed	64	4	6·2
CO_2 as gas from Kipp's apparatus	179	9	5·0

was placed in a large desiccator along with the culture plates and prepared CO_2 was supplied. The preliminary results were so good that they suggested trial under ordinary laboratory conditions. The elaborate sterility precautions were abandoned and a further series of plates was cultured on the same principle as the previous series (table II).

TABLE II

Effect of stabilisation of humidity on the incidence of contaminants

Environment	No. of plates	Contaminants	
		No.	Percentage
CO_2 from acid	92	18	19·6
CO_2 from acid; acid container removed	170	20	11·8
CO_2 as gas from Kipp's apparatus $+$ LiCl	298	2	0·7

Result statistically significant ($P = 0·001$).

These results showed that the abandonment of sterility precautions was associated with a distinct but relatively constant increase in the incidence of contaminants under ordinary conditions but that contaminants were almost completely eliminated by LiCl-controlled humidity. Gonococcal cultures were unaffected.

Summary

Recurring contamination of gonococcal plate cultures with an organism resembling *Bacillus cereus* could not be controlled or eliminated by any of the usual procedures. Stabilisation of the humidity of the atmosphere in which the cultures were incubated by the use of a saturated solution of lithium chloride proved effective.

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616—032 . 021 . 6 : 611 . 778 : 576 . 893 . 192 . 6
(Plasmodium cynomolgi)

THE FATE OF SPOROZOITES OF *PLASMODIUM CYNOMOLGI* INJECTED INTO THE SKIN OF *RHESUS* MONKEYS

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and

T. SOMMERVILLE

The experiments on which this paper is based were originally an attempt to find exo-erythrocytic forms of a malarial parasite in monkeys by repeating the experiments of Huff and Coulston (1944) which were successful in birds.

Huff and Coulston found that sporozoites of *Plasmodium gallinaceum* injected into the wing skin of chickens entered large mononuclear cells resembling histiocytes and there developed schizonts. Two generations of asexual development were thus passed before overt blood infection began. This observation seemed to provide an answer to the mystery about what happened to malarial parasites during the incubation period : the parasite was undergoing schizogony in cells of the reticulo-endothelial system. We repeated these experiments, using *P. cynomolgi* in *Macaca mulatta*, trying not to lose sight of the sporozoites after injection. Huff and Coulston (1947) later did the same thing and were equally unsuccessful. Our experiments, however, are worth describing, as they throw some light on the means by which the mammalian tissues dispose of living particulate matter.

Experimental procedure

Sporozoites were obtained by grinding up the bodies of infected mosquitoes (*Anopheles annularis*) in monkey serum ; about 50 bodies to 0·25 c.c. This dose was equally divided and injected subcutaneously into two areas on the abdominal skin of a monkey, which was named the " donor." Nineteen monkeys were so treated. Three sets of observations were made :—

1. After an interval ranging from seconds to minutes, hours or days, one of the inocula was excised and fixed in formol-saline for histological examination.

2. After a similar interval the other inoculum was excised and placed in the subcutis of an uninfected monkey, which was named the " recipient." In this way we hoped to find how long viable sporozoites remained in the skin inoculum.

3. At various intervals, anything up to 20 c.c. of heart blood was removed from the donor monkey and injected, usually intravenously, into a second uninfected monkey to see whether it would develop malaria, thus indicating the presence of sporozoites in the systemic blood of the donor.

Results

(For the full tables, see Lloyd, 1948)

1. Sporozoites were found histologically in the skin inoculum in every one of 12 biopsies taken up to two hours after injection and in none of 7 biopsies taken 4 hours to 8 days after injection.

2. The recipient monkey developed malaria after receiving inocula of the following ages : 2, 4, 6, 8, 10 and 30 minutes, 1 and 2 hours ; and failed to

develop malaria after receiving inocula 15 and 20 minutes and 4, 8 and 16 hours and 1, 2, 3 and 8 days old. In similar experiments, Hawking (1948) was able to see sporozoites at the site of inoculation up to 4 hours 20 minutes after injection.

3. Evidence of circulating sporozoites was obtained only twice in 22 blood subinoculations done within the first day of sporozoite inoculation into the donor monkey. These were after intervals of 20 and 40 minutes ; 7 of the 22 were done within the first 5 minutes of sporozoite inoculation and were all negative. After 9 days, of course, the blood of the donor became infective as a result of the circulating erythrocytic parasites.

Morbid anatomy. For us the interest in these experiments lies in the way in which the sporozoites disappear. The crucial specimen was that removed after 2 hours, because not only was this the last one to show sporozoites, but it was also the last to be infective for another monkey on subinoculation. Moreover it had an unusually long incubation period in the recipient monkey—31 days instead of the usual 15 ± 6 days.

This biopsy after two hours shows separation of the inoculum into its liquid and solid components. The liquid can easily be identified owing to its content of melanin granules derived from the mosquito's fat-body. Superficial and deep to the inoculum there is a brisk inflammatory-cell infiltration, consisting chiefly of neutrophil leucocytes, which are invading the inoculum. But whereas sporozoites were easy to find in large numbers in the uninvaded portion of the liquid part of the inoculum, very few could be seen in the portion which had been invaded by inflammatory cells.

All the evidence suggests that the sporozoites are killed at the site of inoculation between 2 and 4 hours after injection, probably by some lytic action of the leucocytes, since phagocytosis of sporozoites could not be seen to occur, and that infection of the blood stream must take place before about $1\frac{1}{2}$ hours. By this time histological examination shows the inoculum to be effectively walled off by the inflammatory-cell infiltration. The unusually long incubation period in the recipient after two hours may be due to " sickness " of the sporozoites, which had been almost killed in the body of the donor.

Discussion

The question arises : do the sporozoites leave the site of inoculation by the blood vessels or by the lymphatics ? Though we did no experiments to prove it, we must assume that they left by the lymphatics. Inflammation renders the capillary endothelium quickly semi-permeable, but not to bodies of the size of sporozoites $(12 \times 1 \mu)$. Moreover, the movement of sporozoites is not directional ; it only helps to prevent the sporozoite from sticking to contiguous objects, so that there is no good reason for assuming that sporozoites can enter the capillaries from the tissue spaces. Besides, this is not the most usual method by which the tissues get rid of œdema fluid and particulate matter. Barnes and Trueta (1941) showed that substances of molecular weight 20,000 or more, including bacteria, travel from the tissues to the blood only by the lymph stream. They also showed that inflammatory œdema greatly increases the flow of lymph, which begins in a few minutes and is maximal after 1 or 2 hours, depending on the activity of the part. It is probable, therefore, that sporozoites will be absorbed by the lymph channels in the same way as bacteria, reaching the blood by way of the thoracic duct. Technical difficulties prevented us from demonstrating sporozoites in the lymphatic fluid by cannulating this duct. The results of blood subinoculation are in conformity with this hypothesis.

These observations must not be taken to have any bearing on the question of what happens to the sporozoites when the mosquito bites. Gordon and Lumsden (1939) watched *Aëdes ægypti* taking blood from the web of a frog's foot. Sometimes it plunged its proboscis straight into a capillary, in which

case the feed was rapidly completed; at other times it lacerated a capillary and drew blood from the pool which formed in the connective tissue. Whether the same process takes place in the monkey bitten by an *Anopheles,* we do not know. However, if the blood vessel is indeed cannulated by the anopheline proboscis, the sporozoites will gain direct entry to the blood stream. Fairley's (1945) observation that the blood is infective at the time of biting supports this view. If, on the other hand, the mosquito feeds from a pool of blood, the sporozoites will gain entry by way of the lymphatics, assisted by the inflammatory œdema caused by the irritant action of the mosquito's saliva. This method would be analogous to our injection by needle, which provided no evidence of sporozoites circulating in the blood stream immediately after injection.

Conclusions

Experiments to follow the fate of sporozoites derived from ground-up mosquitoes injected into the subcutis of monkeys showed that no sporozoites remain in the inoculum for more than 4 hours. We give reasons for concluding that they reach the general circulation by the lymphatics, any remaining at the site of inoculation after about 2 hours being killed by the inflammatory reaction. It must not be taken that this conclusion applies to sporozoites injected into the skin by the bite of a mosquito, in so far as the route by which they reach the general circulation is concerned.

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616 . 5—006 . 468 : 599 . 742 . 4 *(Putorius putorius)*

MULTIPLE SQUAMO-SEBACEOUS CARCINOMAS OF THE SKIN IN A POLECAT, *PUTORIUS PUTORIUS*

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Zoological Society of London

The subject was an adult male, acquired in August 1942, which died in June 1948, *i.e.*, aged at least six years. It was noticed to have sessile ulcerated tumours, crusted with blood, on the outer side of the right hind leg, the back of the neck and under the tail near the root. The largest was about 4 cm. in diameter. They were all quite mobile and appeared to be attached only to the skin and not to deeper structures. The animal was long-haired and shy

and its keeper was unable to say for how long the tumours had been present. Surgical removal of the leg tumour was attempted, but so large a raw surface would have been left that it was decided to destroy the animal at once.

Sections of the tumours showed the main background to consist of squamous cells with marked development of cell nests. At the surface there had been much ulceration in places, with hæmorrhage and infection. A well-marked formative layer of the rete was present beneath the surface and this appeared to differentiate into cells of two types, one squamous, forming large sheets of cells with well-marked keratinisation in places, the other resembling exactly the cells of normal sebaceous glands. These formed rounded areas arranged in groups surrounding roughly-demarcated spaces like the central areas of normal sebaceous glands. Many cells intermediate between these types were evident near the formative layer.

Many cells of both squamous and sebaceous type, but especially the latter, were in process of mitotic division, and from this, as well as the irregular nature of the growth, it was clear that the tumour was malignant.

616 . 564 : 599 . 323 (*Sigmodon hispidum*)

SWEAT-GLAND TUMOUR IN A COTTON RAT, *SIGMODON HISPIDUM*

R. E. REWELL

Zoological Society of London

The subject, an adult female cotton rat, was born in the Zoological Gardens on 30th June 1946 and died there on 28th August 1948. At necropsy, extensive bilateral bronchopneumonia was found, with a large abscess of some age in the upper anterior mediastinum, cultures from which yielded *Proteus vulgaris* and *Staphylococcus albus*. There was also what was mistaken for an enlarged lymph node in the right inguinal group. This was ovoid, some 2 cm. long, and of a uniform, rather fatty consistency. It lay superficial to the lymph nodes and just beneath the skin, to which it did not appear to be attached, nor did it invade any other structures.

Section of the mass revealed a tortuous adenoid structure composed partly of tubules lined with epithelium, but mainly of papilliferous processes clothed with a single layer of epithelial cells applied to a fine fibrous stroma richly supplied with blood vessels. In a few places the epithelium was several layers thick and even one or two sheets of cells were formed. The epithelial cells were uniformly cuboidal, with rather large pale nuclei. Hæmorrhage had occurred in several places and here many macrophages were found, some of them laden with blood pigment.

The tumour would appear to have been benign or of only low malignancy. Many sweat-gland tumours have been reported from dogs, but not from rodents, although these animals do possess sweat glands. In the present case the site of the tumour is on an extension of the " milk line " and so the primary site may have been a small mammary gland rather lower down the body than usual for the species. No nipple was noticed there at necropsy. It seems best however, to assign the tumour provisionally to the less specialised sweat gland than the more specialised mammary gland.

616—006 . 447 : 616 . 61—003 . 811

A CASE OF DIFFUSE PLASMOCYTOSIS WITH DEPOSITION OF PROTEIN CRYSTALS IN THE KIDNEYS

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(PLATES XXXI-XXXIII)

IT has long been known that multiple myeloma in a majority of cases is attended by peculiar disturbances in protein metabolism. Kahler (1889) is given credit for having been the first to point out the coincidence of Bence-Jones proteinuria with the disease which now bears his name. Although some doubts were expressed later as to the specificity of Bence-Jones proteinuria, it seems, according to Apitz (1940*b*), that most of the instances which have been brought forward against it were in fact cases of unrecognised or misinterpreted myeloma. It must be kept in mind that (1) the cells of myeloma may differ considerably from typical plasma cells owing to blastomatous anaplasia, and (2) the existence of diffuse plasmocytosis without manifest tumour formation has not been recognised until recently.

Bence-Jones proteinuria, however, is not the sole manifestation of disturbed protein metabolism. Perlzweig *et al.* (1928) showed that in most cases of myeloma there is an increase in blood proteins—a hyperproteinæmia. Magnus-Levy (1931-1937), who in a series of papers dealt with the various aspects of metabolic disturbance in myeloma, pointed out that while hyperproteinæmia may be indicative of myeloma, there are in fact several diseases in which an increase in plasma protein may be found, particularly kala-azar. The amount of plasma protein in myeloma may be as high as 18·37 per cent. (Foord, 1934-35) instead of the normal 7 or 8 per cent.

As to the component of the plasma proteins responsible for this rise, Magnus-Levy found it to be mainly euglobulin ; several observers,

however, found it to be pseudoglobulin. Magnus-Levy expressed the view that the increase is the result of an over-production of normal serum proteins in the bone-marrow.

Later, substantial objections were raised to this conclusion, as von Bonsdorff *et al.* (1937) found in the serum of their patient a protein which differed in many respects from all normal components, and particularly in its self-flocculation and crystallisation; it was considered by these authors to be foreign to the blood. A similar conclusion had been reached by Wintrobe and Buell (1933) on the basis of the antigenic properties of the protein isolated from a patient with myeloma. This particular substance, however, is by no means identical with Bence-Jones protein, its molecular weight being much higher. As a matter of fact, Bence-Jones protein has so far been demonstrated in the blood only on rare occasions and then only in small quantities, certainly not sufficient to account for the high-grade hyperproteinæmia.

Another aspect of the disturbance of protein metabolism is the rather frequent occurrence of atypical amyloidosis in myeloma: something like 40 cases of this association are on record. For this, Picchini and Fabris (1930) coined the term paramyloidosis, a term which was introduced into the German literature by Strauss (1933).

In 1940(*b*) Apitz submitted the whole question to a very thorough analysis, based on biochemical as well as morphological data. He concluded that the tumour cells of myeloma themselves produce a whole range of abnormal proteins, all of which are " blood-foreign." Owing to this quality, they are excreted by the kidneys, partly as Bence-Jones protein, itself by no means a single well-defined substance, partly as proteins of higher molecular weight. In other cases the abnormal protein is deposited in the tumour or in various organs in the form of paramyloid. In Apitz's opinion the plasma cells in general are the producers of the serum proteins, while in myeloma this function is disturbed to the point of giving rise to pathological products. For this disturbance he suggests the term paraproteinæmia, while the excretion of these proteins by the kidneys he calls paraproteinuria. Some years later Brass (1942-43*a*, 1944) dealt with this subject in much detail. He came to almost identical conclusions to those of Apitz, though differing in some minor points. In one of his cases there was Bence-Jones proteinuria without hyperproteinæmia; this of course did not mean that the blood proteins were qualitatively normal.

Both Apitz and Brass, attacking the problem from the morphological point of view, were successful in demonstrating certain histological facts, hitherto unknown, which they interpreted as indicating paraproteinæmia in addition to or in the absence of paramyloid. In the tumour cells of some cases they found acidophilic droplets, as had previously been reported by Parkes Weber (1903). Globular bodies were seen by Brass lying free amongst the cells and

also in the plasma within the vessels. He further reported droplet-like masses inside the stellate cells of the liver. All these structures resembled Russell's bodies or hyaline droplets, as seen in the epithelium of the kidneys, both in shape and in staining properties. In Apitz's view they are best accounted for by the colloidal process of coacervation (Bungenberg de Jong, 1932), and Brass actually gives a picture of coacervates of Bence-Jones protein in the urine—the "globulites" of Magnus-Levy—which are very similar to those he found in his microscopical sections. The particular staining properties, which include acidophilia and selective staining by a modified Weigert's method for fibrin, do not indicate chemical identity but a similar colloidal condition.

Still more interesting though much rarer is the finding of crystalline material microscopically. Intra- and extracellular crystals were first seen in a myelomatous tumour by Glaus (1917), while in Abrikossoff and Wulff's case (1927) they were situated outside the cells only. Steinmann (1939-40) found crystalline inclusions in tumour cells in a sternal marrow biopsy from a case of leukæmic plasmocytoma. Apitz (1940b) described a similar finding in two of his cases (1613/39 and 314/45), the second of which was one of diffuse plasmocytosis. Brass reported three instances of intracellular crystals in tumour cells and in two further cases crystals were found, not in the plasmocytes themselves, but lying loose in their midst as well as in histiocytes and fat-cells, and inside the blood-vessels of the bone-marrow. It is to be noted that crystalline inclusions in plasmocytes do not occur exclusively in myeloma but have been seen also in rhinoscleroma by Freifeld (1913) and others.

Still rarer seem to be crystalline deposits outside the tumours. Brass (1942-43a) described very remarkable instances of this kind in his cases 9 and 12, where there were crystals in the endothelial cells of the lymph-nodes, in the loose fibrous tissue and, in one case, in the stellate cells of the liver.

It is impossible to give here a complete review of the multifarious findings ; for details one must refer to the original papers. We would only add that the crystals behave in the same way as the hyaline droplets to a variety of staining methods and are doubtless of protein nature. Apitz found that they were stained by Congo red, but this was not confirmed by Brass.

In addition to the organs so far mentioned, both hyaline and crystalline deposits may be found in the kidneys as a result of para-proteinuria. It has been known for a long time that in almost all cases of myeloma with Bence-Jones proteinuria the kidneys show damage of differing degrees. Many patients with myeloma die of uræmia. Since von Decastello (1908-09) gave the first description of the "myeloma kidney," a series of papers has been published on this subject. In advanced cases the kidneys show much fibrosis and shrinkage, which Bohnenkamp (1922) and others ascribed to blocking

of the tubules by casts of special appearance. In view of this assumption, which makes the process akin to hydronephrosis, with the difference, however, that the lesions are focal in character, Ehrich (1932) introduced the term nephrohydrosis. Perla and Hutner (1930), on the contrary, stated that the shrinking was due to concomitant arteriosclerosis ; this view, however, is \not shared by any of the other workers in this field. The casts are often surrounded by foreign-body giant cells, a phenomenon which is uncommon with casts of other origin and is indicative of the pathological quality of the protein excreted.

The contracted kidney, however, represents an advanced stage of the disease. In more recent cases changes are seen which Randerath (1934-35) has classified as nephrotic. The epithelial cells are filled with hyaline droplets and the basal membrane of the glomerular tufts may show thickening. There is little doubt to-day that the droplets, which are also seen in glomerulonephritis, ordinary amyloidosis and common nephrosis, are due to excretion by or filtration of protein through the glomeruli and to its partial resorption by the epithelial cells of the tubules. It must be stressed that notwithstanding their origin they stain in the same way as in Bence-Jones albuminuria. Randerath believed that the formation of casts was secondary to the glomerular lesions, which tended to check glomerular filtration. This was opposed by Apitz, who thought that the casts were produced, in part at least, by primary coacervation of the protein excreted.

Cases in which the casts had a definitely crystalline character, have been observed much more rarely. The first observation of that kind is credited to Löhlein, who in 1913 showed the slides of his case at the meeting of physicians and scientists in Vienna but published his paper only in 1921. Up to the present 13 cases only have been recorded (*vide infra*). It seems, however, that the lesion is not so rare as may appear from the scarcity of published cases. Apitz saw it twice in 18 cases of myeloma and Brass twice in 15 cases. From these figures one may infer that the appearances have often been overlooked by others. This may warrant the description of a case of the kind, in spite of the fact that we have given the pathology of myeloma in general far less thorough study than either Apitz or Brass. In none of the seven additional cases of multiple myeloma or plasmocytosis which we have studied since could we find crystals in the kidneys.

CASE REPORT

R. S., a 57-year-old charwoman, was first admitted to the hospital of the District Health Insurance Association on 7.3.41, 3 years before she died. Her main symptoms were due to an acute respiratory infection, with broncho-pneumonia which cleared up within a week. In addition to the acute illness, rather severe anæmia was noted and a systolic murmur was heard over the apex of the heart. The patient was discharged after a week's stay but was instructed to report again in a fortnight.
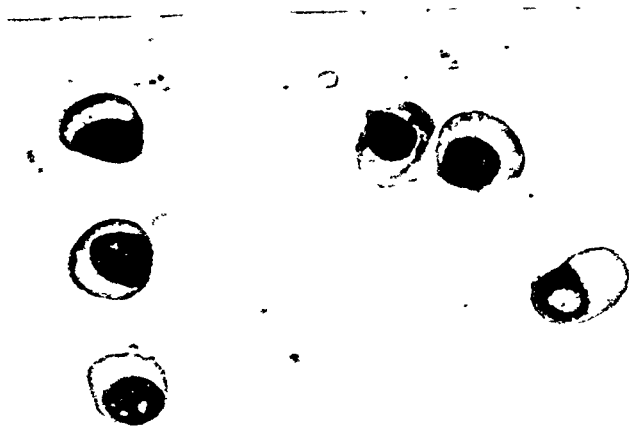
DIFFUSE PLASMOCYTOSIS

FIG. 1.—Plasma cells in smear from sternal puncture.   Giemsa's stain.   ×600.
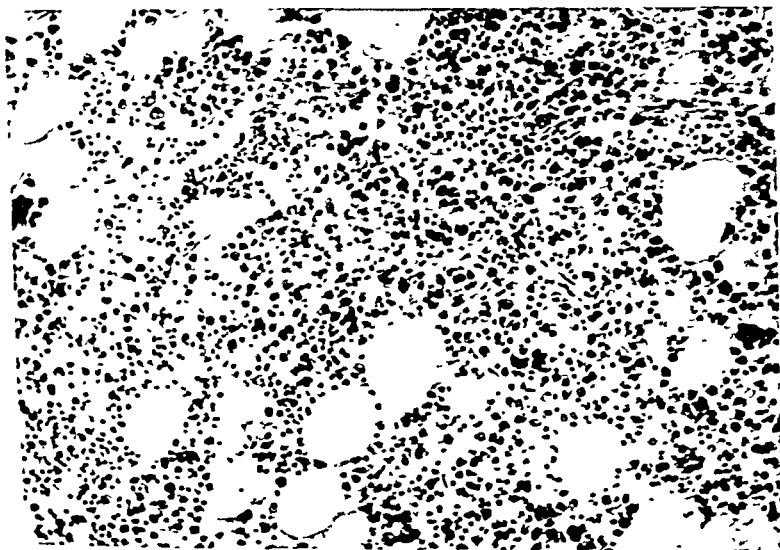

FIG. 2.—Bone-marrow of femur showing diffuse infiltration with plasma cells.
Methyl green-pyronine.   ×220.

On the second admission (31.3.41) the blood-count was 2·1 million red cells with colour index 1·07. A sternal marrow biopsy was also made, but the report has unfortunately been lost. After her discharge she was treated in the out-patient department until 1944 with injections of a liver preparation. The red cell count showed some improvement at first, the red cells rising to 3·5 million in 1942. Later, the course was slowly but steadily down hill, and on 12.5.44 the red cells fell to 0·66 million, and the colour index rose to 1·38. During the whole time the white blood-cells showed no particular changes except for eosinophilia, reaching at one time 15 per cent. Plasmocytes were never recorded. The patient was once more admitted to the in-patient department owing to her complaint of weakness and tiredness, dyspnœa and coughing. A sternal puncture made on 24.5.44 showed almost exclusively plasmocytes of rather typical form (fig. 1). A tentative diagnosis of myeloma was made, although no tumours could be found on X-ray examination. The condition of the patient grew steadily worse and she developed œdema and albuminuria. From 26.6.44 on several occasions Bence-Jones proteinuria could be demonstrated. The patient was given several blood-transfusions, but these brought no improvement, and she died on 7th July 1944, at the age of 57.

Clinical diagnosis :—Hyperchromic anæmia : myeloma ?

Post-mortem examination

The post-mortem (1190/44) was performed 24 hours after death by Dr Benešová. The body was that of an elderly, slender, rather emaciated woman. Both lower extremities were œdematous and showed several hyperæmic areas on the shins, where the epidermis was sloughing off in big scales. There was also some œdematous swelling of the neck. The sclera and the upper part of the body showed low-grade jaundice.

There was nothing particular about the cerebral cavity and its contents except for definite anæmia of the *brain.* No tumours were found in the skull, but the *bone-marrow* appeared rather dark red.

The *lungs* were œdematous and the bronchi contained frothy serous fluid. In the right middle lobe there was a calcified primary tuberculous lesion and the regional lymphatic gland in the hilum was also partly calcified.

The right *heart* showed definite hypertrophy, the muscle of the right ventricle being up to 6 mm. thick. The weight was 580 g. There was a patent foramen ovale 3 × 2 cm. wide. There was some thickening of the aortic cusps. Post-mortem coagula were rather bulky in the right heart and had the usual appearance. The *tongue* was fairly normal, the *tonsils* slightly scarred, and some lacunæ contained plugs.

The *spleen* was considerably enlarged (780 g.) On section the tissue was dark red with a brownish hue, and rather firm.

The *kidneys* were of normal size, weighing together 360 g. Their surface showed hardly noticeable granulation. On section the cortex was brown-red with some greyish stripes and somewhat transparent ; the pyramids were dark red, otherwise normal.

No special lesions were found in the rest of the *urogenital system, intestine* or *pancreas.* The *gastric mucosa* showed an average grade of autodigestion. The *gall-bladder* was bulky and contained a quantity

of dark brown bile. In the mucosa were some whitish streaks, due to lipoid deposits.

The *liver* was fairly large (1700 g.), with some gross lobulation on the surface but little scarring on section. The parenchyma was yellowish brown with a rusty tint.

The *aorta* and larger arteries contained a few arteriosclerotic plaques.

The *bones* were definitely porotic, with their corticalis rather thinned. The ribs broke readily. The *bone-marrow* in all parts examined (sternum, ribs, several vertebræ and left femur) was cherry coloured. No tumours were found anywhere.

The results of the post-mortem examination were rather disappointing, since the expected multiple myelomata were not found. On the other hand, the findings did not quite conform to Addison's anæmia, as there was no atrophic gastritis or Hunter's glossitis. It was only on microscopic examination that the real nature of the condition became apparent.

Histology

Bone-marrow. In the cancellous bones the bone-marrow is almost entirely cellular, containing a small number of fat-cells only, whereas in the femur fat-cells are rather numerous. The bulk of the cellular tissue lying among them consists of fairly uniform cells, polygonal, rounded or oblong, with round or slightly oval nuclei, situated eccentrically (figs. 2 and 3). The average diameter of the cells is 9 μ, the diameter of the nuclei ranging between 4 and 7 μ. A few cells contain two nuclei. The cytoplasm is highly basophilic, staining dark violet with Giemsa's solution in Zenker-fixed material. In alcohol-fixed material stained with Unna-Pappenheim's methyl green-pyronin the cytoplasm appears deep red. In many of the cells an ill-defined unstained area is seen on one side of the nucleus. With higher magnification the cytoplasm is found to be studded with vacuoles of variable size, the contents of which stain pink with eosin. The nuclear membrane is thick, and the chromatin appears as coarse particles which are often contiguous to the membrane. These particles are mostly triangular in shape, though the typical spoked-wheel arrangement is well marked in only a small proportion of the cells. Some cells have relatively bulky nuclei with less coarse chromatin particles; such cells are not easily differentiated from the most primitive marrow cells. Nucleoli are rarely seen, being largely masked by the chromatin; they are generally single and rather large, staining pinkish grey with hæmatoxylin and eosin and reddish with Unna-Pappenheim. No Russell bodies or other acidophil inclusions are seen, and the Weigert-Pfister method gives a completely negative result.

The cells so far described are diffusely and rather evenly distributed in all parts of the bone-marrow examined; nowhere is there definite
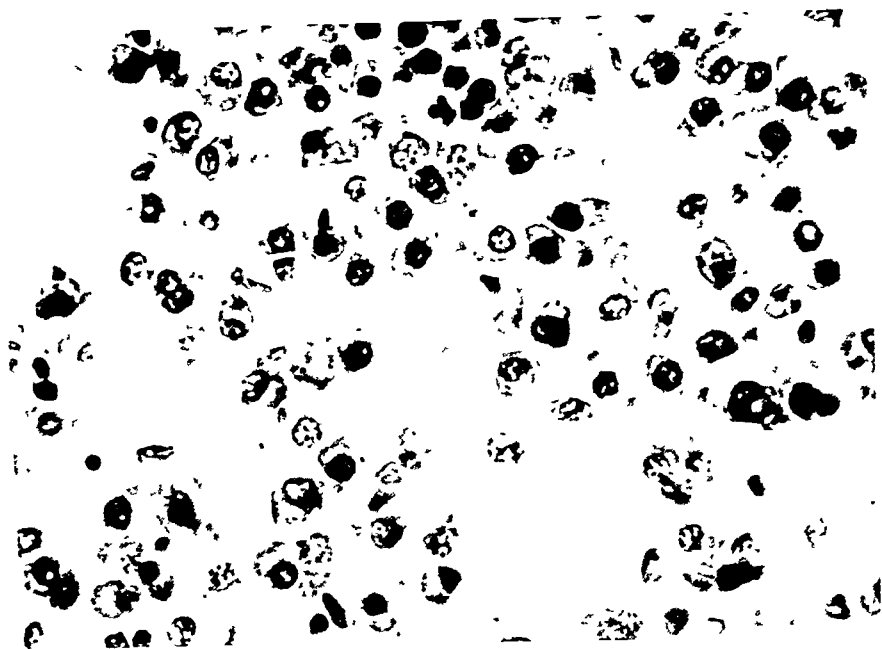
DIFFUSE PLASMOCYTOSIS

FIG. 3.—Bone-marrow of femur. Atypical plasma cells. Methyl green-pyronine.
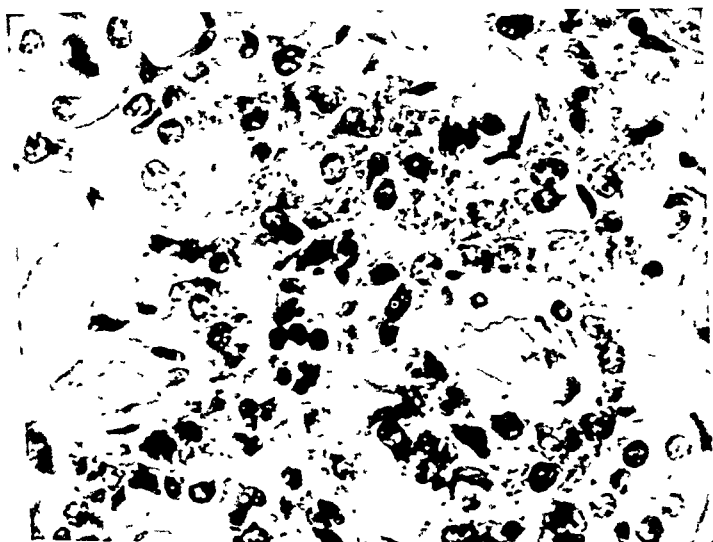×580.

FIG. 4.—Nest of myeloma cells from the renal medulla. The minute crystals in their
cytoplasm, well shown in the stained section, are not readily made out in the
photomicrograph. Weigert-Pfister stain. ×580.

tumour formation or even any tumour-like accumulation. Scattered amongst the plasma cells are foci of normal blood-forming elements of both the red and white cell series. Very few megakaryocytes are encountered.

Spleen. The pulp cells are swollen ; numerous partly decolourised red cells are seen engulfed in their cytoplasm, and there is also a rather large amount of· iron-positive blood pigment. The sinuses contain a good many foci of erythropoiesis. Atypical plasma cells are also seen, lying mostly inside the sinuses, singly or in small groups. The small arteries show some hyalinisation. Lymphoid follicles are small and scanty.

Lymph-nodes (mesenteric and subtracheal). The sinuses are much widened and mostly filled with proliferated endothelial cells mixed with erythrocytes. There are also a few granular cells resembling myelocytes, but no erythroblasts. The pulp cells are rather atrophic and secondary follicles are absent. In some of the strands there are fairly large groups of basophilic cells similar to those in the bone-marrow except that they show greater polymorphism, particularly of the nuclei, some of which are much larger. Binucleated cells are also more numerous than in the bone-marrow. In some places the accumulations of these cells appear almost like tumours of microscopic size. In the sinuses only some isolated cells of this kind are encountered. More frequently ordinary plasma cells are seen and there are a few cells with round pyknotic nuclei the size of a lymphocyte, the bulky cytoplasm of which is stuffed with eosinophil granules. In one section stained with hæmatoxylin and eosin a peculiar cell is found in a pulp strand amid an accumulation of the pathological cells ; this cell, probably a histiocyte, has an eccentrically placed pale nucleus, and the cytoplasm is filled with very minute pink-staining crystalline granules. This finding, however, cannot be duplicated in any other section. The Weigert-Pfister method failed to show anything noteworthy.

In the subtracheal nodes several small foci of calcification are seen, obviously healed tubercles.

Liver. The lobular arrangement is somewhat irregular, even apart from the fibrous strands. There are patches of moderate fatty change irregularly distributed but mainly centrolobular. The liver cells contain much finely granular iron-positive pigment. The sinusoids are engorged and their walls slightly thickened, and the peri-capillary spaces of Disse are clearly seen everywhere. The endothelial and stellate cells are somewhat swollen : few of them contain iron-positive pigment. In some sinusoids groups of erythroblasts are seen. A fair number of isolated atypical plasma cells are also encountered, freely suspended in the blood of the sinusoids. The Weigert-Pfister stain does not disclose any foreign material in the Kupffer cells or elsewhere.

Kidneys. The general pattern is fairly well preserved but the interstitial tissue is diffusely increased, particularly in the medulla.

In the cortex there are some poorly defined areas of tubular atrophy, with accentuation of the fibrosis, mostly corresponding to individual nephrons. Single glomeruli are completely hyalinised. The interstitial tissue is rather acellular except for some minute accumulations of lymphocytes in the areas of atrophy and a few foci of erythropoiesis in the medulla. There is low-grade thickening of the intima in the middle-sized arteries and slight focal hyaline change in some arterioles.

The majority of the glomeruli appear almost normal except for very slight thickening of the basal membrane of the tufts and the fibrous capsules ; there is also some swelling of the epithelium of both glomeruli and capsules. The capsules are somewhat distended and contain finely floccular, faintly staining material.

With the low power the proximal convoluted tubules outside the atrophic foci appear little changed, but the cytoplasm of the epithelial lining is highly granular. The lumina of most of the tubules contain floccular masses similar to those in the capsules. Many of the loops of Henle and the distal convoluted tubules are distended with a pathological substance, composed of polymorphonuclear leucocytes in rare instances, but mostly of finely granular detritus or hyaline casts. The latter have a glassy, highly refractile appearance ; with Masson's trichrome they stain more or less deep blue, and some of them contain bright red inclusions.

What renders the lesion particularly striking, however, is the presence of crystalline deposits in some of the tubules. These are inconspicuous in sections stained with hæmatoxylin and eosin, in which they are coloured pink : by the van Gieson method they are stained yellow and again are indistinct. With Masson's trichrome, on the other hand, they are very prominent, being stained bright red (fig. 5). The best method for their demonstration, however, proved to be Pfister's modification of Weigert's stain for fibrin. With this method all the crystals acquire a deep violet colour (fig. 6). In polarised light a faint double refraction is noticed in some of the larger crystals.

The renal tissue had been fixed in formol-Müller. In other blocks. fixed in alcohol the contours of the crystals appear smudged. This, in our opinion, is due to incomplete fixation of the protein, which later, on staining, had been partly dissolved. The crystals, however, are certainly not soluble in alcohol.

Single crystals have the shape of thin prismatic plates, which for the most part are packed closely together. As they are generally cut transversely, they usually appear as thin needles, but in moving the micrometric screw one clearly notices that they are in fact plates. Their size shows much variation ; the biggest are up to 80 μ long and 30 μ broad. Often they are bent or otherwise deformed which is probably due to dehydration of the tissue. The edges sometimes show irregular erosion. Some isolated crystals, bigger and better preserved, appear on cross section as narrow prisms with flat pyramids at both ends.
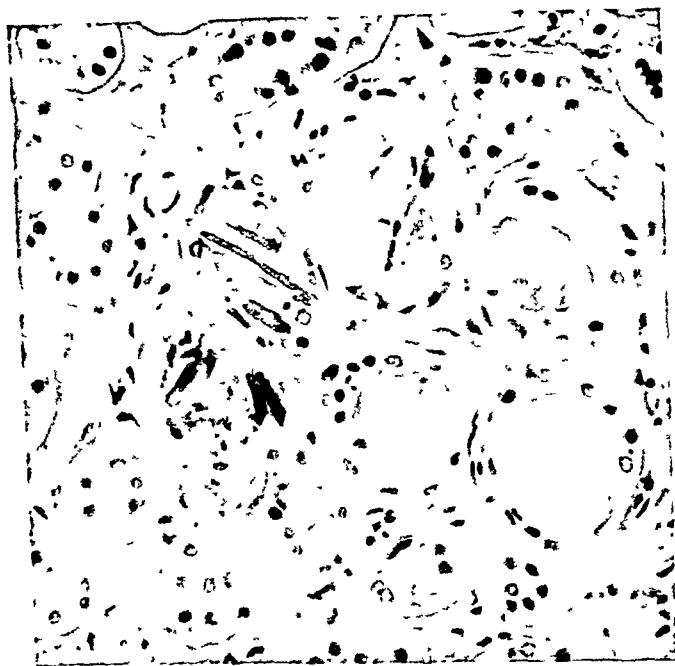
DIFFUSE PLASMOCYTOSIS

FIG. 5.—Protein crystals inside the tubules of the kidney. Masson's trichrome stain. Kodak Ectachrome film. ×320.

FIG. 6.—Protein crystals in the cytoplasm of the epithelium of the proximal convoluted tubules. Weigert-Pfister stain. Kodak Ectachrome film. ×270.

Some of the crystals lie singly or in groups inside tubules which contain no other material. For the most part, however, the crystals are surrounded by detritus, more rarely by leucocytes, or are embedded in casts. Their position in the nephrons coincides with that of the casts, that is to say, they were found in the loops of Henle and rarely in collecting tubules, but most of them are in the distal convoluted tubules. The epithelium of tubules that contain detritus, casts or crystals is flattened or detached from the basal membrane ; rarely it is entirely missing. In other involved tubules it has proliferated slightly. Giant cells are encountered on rare occasions.

In addition to those seen in the lumen, similar though usually much smaller crystals are found inside the epithelial cells lining the proximal tubuli contorti. They are seen in sections stained by Masson's method, but are mostly masked by other cytoplasmic granules staining grey. It is only after Weigert-Pfister staining that the extent of this lesion can be appreciated (fig. 6). The size of the crystals is very variable. Some nephrons contain minute specimens on the verge of visibility ; in others they are both much larger and more numerous. Sometimes a single cell appears to be stuffed with crystalline material. Here the shape of the crystals is rather that of thin needles, but small prisms are also seen. No crystals or other Weigert-staining corpuscles are found in the glomeruli.

In some sections stained in this way small groups of neoplastic plasma cells are encountered in the medulla ; their cytoplasm, too, is filled with very tiny needle-shaped crystals (fig. 4).

Discussion

The microscopical examination left no doubt that this was a case of diffuse plasmocytoma (plasmocytosis), involving, in addition to the bone-marrow, the internal organs, particularly the liver, spleen and lymph-nodes. Apitz (1940*a*) was the first to call attention to this particular form of overgrowth of bone-marrow plasma cells. He is certainly right in saying that such cases are by no means exceptional but may easily escape attention at the post-mortem.

We have more recently had a similar experience at the autopsy of another case, a woman aged 67, who died of uræmia. Except for some osteoporosis no gross changes were seen in the bones. The kidneys, however, showed fibrosis of unusual appearance which directed our attention to the bone marrow, and on microscopic examination the diagnosis of diffuse plasmocytosis was established. The renal lesion was that of a rather advanced myelomatous nephrosis but without crystalline deposits.

Apitz says that even in a definitely neoplastic myeloma the rest of the bone-marrow often shows diffuse infiltration with plasma-cells. In fact, he believes that diffuse plasmocytosis generally precedes the development of tumours, which, indeed, represent a later stage of the disease.

In rare instances of diffuse plasmocytoma the pathological cells are found in the peripheral blood ; such cases are referred to as plasma-cell leukæmia. In the present case no plasma cells had been reported in any of the numerous blood-counts, although in the post-mortem material they were encountered inside the blood vessels of both liver and spleen.

The most outstanding feature of the present case, however, was the finding of crystalline deposits in the kidneys.

As already mentioned, Löhlein was the first to report this phenomenon in a man aged 50 with multiple myeloma (table). He saw rhomboid crystals inside the tubules of a highly fibrosed kidney. As there was also rather severe Bence-Jones proteinuria, he concluded that the crystals consisted of this particular substance. In 1920-21 Koch made a similar observation in a 44-year-old man with prostatic hypertrophy. In his short communication he did not mention any changes in the bones or bone-marrow. Commenting on Koch's demonstration, Fischer (1920-21) briefly mentioned a similar case, without giving details. Rehsteiner's case (1922-23) was that of a female aged 57 who had suffered from albuminuria and died of uræmia. No test for Bence-Jones protein seems to have been made and nothing was said about microscopic examination of the bone-marrow. Gunn and Mahle (1938) found crystals in a case of what they called megakaryocytic myeloma ; from their microphotographs, however, it appears to have been an atypical plasmocytoma.

Apitz was the first to demonstrate crystals inside the epithelial cells lining the renal tubules. The second of his two cases is interesting in that it was an extramedullary plasmocytoma with paramyloidosis. Hartmann's (1941) two cases were diagnosed as achylic chloræmia and aleukæmic lymphadenosis respectively. Albuminuria was reported in the first case, but tests for Bence-Jones protein were not mentioned in either. The bone-marrow was examined in the second case only ; no plasma cells are said to have been found in Unna-Pappenheim-stained sections. Brass (1942-43a, 1943-44) found crystals in the kidneys of two of his cases, both of them cases of diffuse plasmocytosis. In the first, the crystals were situated inside the epithelial cells as well as in the lumen of the tubules, and also in various cells of the interstitial tissue ; in his second they were in the lumen only. The first case was remarkable also in that the urine tests for Bence-Jones protein were equivocal. In Mücke's (1943-44) first case the diagnosis of chronic nephrosis was made both clinically and at autopsy. No test for Bence-Jones proteinuria had been made. Myelomas, in the author's own words, " were not demonstrated in bones so far examined." Crystals were found in the lumen of the tubules ; they were definitely hexagonal on cross section. Mücke's second case was one of multiple myeloma. The total serum protein was slightly above normal, but the albumin-globulin ratio was much lowered. There had been rather severe albuminuria but tests for Bence-Jones protein were negative on several occasions. The small needle-shaped crystals were mostly intra-epithelial.

On the basis of this survey one may question whether the formation of protein crystals in the kidneys is specific for myeloma, as in 6 of the 13 cases published no myeloma was found *post mortem*. Nor were osteolytic tumours seen in Brass's second case or our own, but microscopic examination of the bone-marrow disclosed diffuse plasmocytosis. Since in the observations of Fischer (1920-21), Koch (1920-21), Rehsteiner (1922-23), Hartmann (1941, 1st case) and Mücke (1943-44,

1st case) the bone-marrow was not examined microscopically, the possibility of these being cases of diffuse plasmocytosis remains open. As a matter of fact, no instance of crystalline deposits in the kidneys has been reported so far in which the bone-marrow proved to be normal microscopically save only the case of extramedullary plasmocytoma seen by Apitz. As to Hartmann's second case, Brass believes it to be an instance of atypical plasmocytosis, the occurrence of which was pointed out by Apitz.

The composition of the crystals is still in doubt. The staining methods used cannot prove much beyond the notion that the crystals are protein in nature. Löhlein thought they consisted of Bence-Jones protein, but there is some doubt about this, since in Apitz's first and Mücke's second case the protein in the urine was of a different kind. As was stated above, the paraproteins isolated from the blood which differ from Bence-Jones substance readily form crystals. It may therefore be admitted that these substances are able to produce crystals in the kidneys as well. On the other hand, the assumption of Brass that the smaller molecules of Bence-Jones protein are split off during the process of excretion cannot be dismissed.

Brass maintains that in one of his cases the crystals were pure Bence-Jones protein because of their hexagonal form, which is characteristic of spontaneous or artificially induced crystallisation of that substance from the urine. However, it seems futile to depend too much upon the type of the crystals, since it is difficult to ascertain their geometric form in microscopic sections with sufficient exactness, and the descriptions given in most papers are rather vague on this point. Moreover the type of protein crystal is, in general, largely dependent on a number of accidental factors such as pH and the presence of mineral salts or other admixed substances.

In our case the crystals were of a rather unusual type, having the form of rectangular plates. Apitz, to whom we sent several slides, states in a personal communication that they are different from those in his own as well as in all the other cases published.

The kidney lesion was relatively little advanced in our case; it certainly did not cause death by itself, the patient dying of anæmia and heart failure. Such instances are certainly more favourable for studying the genesis of the renal condition than the later stages of the same process. The most outstanding feature was the presence of intracellular crystals in the epithelium of the proximal convoluted tubules, a condition which has been observed in a few instances only. We agree with Apitz that the intracellular position of the crystals is best explained by resorption of the pathological protein and not by its secretion *in situ.*

As to whether the renal condition should be classified as nephrosis and not nephrohydrosis seems to us to be a purely academic question, and we are not inclined to stress it as Apitz does. However, it is interesting to note the amount of interstitial hyperplasia, which is

TABLE

List of published cases showing crystallisation out of protein in the kidneys

No.	Author	Year	Case no.	Age	Sex	Clinical diagnosis	Post-mortem diagnosis	Albuminuria	Bence-Jones proteinuria	Site of crystals	Shape	Crystals in other organs
1	Löhlein	(1913) 1921	...	50	M.	Severe anaemia	Multiple myeloma	−	+	Extracellular: convoluted and straight tubules	Rods, rhombi, prisms	...
2	Koch	1920-21	...	44	M.	Chronic nephritis	Syphilitic aortitis (bone-marrow not examined)	?	?	Extracellular: tubules in all parts	Rhombic plates, hexagons	...
3	Fischer	1920-21	...	?	?	?	Nephrolithiasis; no details	?	?	?	?	?
4	Rohsteiner	1922-23	...	57	F.	Progressive weakness; anuria	Contracted kidney (bone-marrow not examined)	+	?	Extracellular: from Bowman's capsules down to collecting tubules	Prisms, rhomboid plates	...
5	Gunn and Mahlo	1938	...	57	F.	Multiple myeloma	" Megakaryocytic myeloma"	−	+	Extracellular: tubuli contorti, loops of Henle, collecting tubules	Rhomboid	...
6	Apitz	1940a	1613/39	66	M.	?	Multiple myeloma	+	−	Almost exclusively intracellular: proximal convoluted tubules	Spindle-shaped	Tumour cells
7	Apitz	1940b	1518/31	53	M.	?	Extra-medullary plasmocytoma with local paramyloid	−	+	Intracellular: proximal convoluted tubules (rare)	Needle-shaped	...

No.	Author	Year	No.	Age	Sex	Clinical diagnosis	Anatomical diagnosis			Localisation	Crystal shape	Other localisation
8	Hartmann	1941	1	65	F.	Hypertonia; "achylic anaemia"	Chronic nephritis	+	?	Extracellular: mostly cortex	Needle-shaped, prismatic	...
9	Hartmann	1941	2	70	M.	Aleukaemic lymphadenosis	Aleukaemic lymphadenosis	?	?	Extracellular: cortex and medulla	Long prismatic or short rhomboid, pebble-shaped	...
10	Bruss	1942-43a	12	73	F.	Panmyelopathia; nephrosis	Diffuse plasmocytosis	+	±	Intracellular: proximal convoluted tubules, loops of Henle	Polymorphous, mostly rhomboidal with curved edges, almost elliptical or globular. Rhomboid, double pyramids, sume	Interstitium, reticulum and fat-cells of bone-marrow
11	Bruss	1943-44	Sch.	68	M.	Generalised plasmocytic myeloma	Predominantly diffuse plasmocytoma	+	+	Extracellular: mostly loops of Henle and interstitium	Rhombohedric, polygonal, hexagonal	Bone-marrow; duct of salivary gland
12	Mieke	1943-44	1	50	M.	Chronic nephritis	Severe nephrosis (bone-marrow not examined)	+	?	Extracellular: proximal and distal convoluted tubules, loops of Henle	Needle-shaped, quadrangular, hexagonal	...
13	Mieke	1943-44	2	50	M.	Multiple myeloma	Multiple myeloma	+	–	All tubules	Small needles	...
14	Personal case	1949	...	57	F.	Hyperchromic anaemia, multiple myeloma (?)	Diffuse plasmocytosis	+	+	Mostly intracellular. Intracellular: proximal convoluted tubules. Extracellular: loops of Henle, distal convoluted tubules, collecting tubules	Small thin plates. Prismatic plates	...

out of proportion to the relatively small number of nephrons blocked by casts or crystals ; this is difficult to explain. The arteriosclerotic changes in our case were negligible.

In other parts of the body, including the bone-marrow, we failed to demonstrate crystals except for one rather questionable instance in a lymph-node. Nor could we find any substances staining with Weigert's method. This particularly applies to the plasma cells themselves, with the exception of a small focus in the medulla of the kidney, the cells of which contained tiny Weigert-positive crystals. A possible explanation of this finding could be the assumption that the interstitium of the kidney was soaked with the resorbed pathological protein, which was stored by the plasma cells. Brass in one of his cases found crystals in the fibroblasts of the kidney. As, however, the sections containing the crystals came from a block of tissue which had been fixed in formol-Müller, whereas the bone-marrow and other organs had been fixed in Zenker and alcohol only, we are not inclined to give this isolated finding undue importance. The presence of foreign protein in the interstitium of the kidney may perhaps also account for the fibrous tissue hyperplasia.

Summary

In a female aged 57 who had been suffering for 3 years from progressive hyperchromic anæmia resistant to liver therapy, sternal puncture disclosed the presence of plasma cells and tests for Bence-Jones proteinuria were positive. A tentative diagnosis of multiple myeloma was made, although no tumours could be demonstrated by X-ray examination.

At post-mortem, diffuse plasmocytosis of the bone-marrow was found, with foci of the pathological cells in the spleen, liver, kidneys and lymph-nodes. The kidneys showed a particularly interesting condition, in that many of the tubules and most of the epithelial cells of the proximal convoluted tubules contained protein crystals. This is the 14th case in which this finding has been recorded, though not in all of them was the presence of myeloma established.

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MULTIPLE PLASMA - CELL MYELOMA WITH CRYSTALLINE DEPOSITS IN THE TUMOUR CELLS AND IN THE KIDNEYS

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(PLATE XXXIV)

THE following is a short account of a second recent case of plasmo-cytic neoplasia, occurring in Czechoslovakia, in which enormous crystalline deposits of protein were present in the kidneys.

CASE REPORT

Clinical history

A. P., a male aged 45 years, was admitted on 14th October 1948 to the medical clinic of Professor Stejfa, to whom I am indebted for the clinical data. The family history was without significance. The patient had suffered from rheumatic disease since his 11th year. Later, from the age of 28, he had tonsillitis every year. The present illness started in May 1948. Since that date he had been developing progressive tiredness, weakness and sleepiness, with loss of weight. From September he suffered from dull pain in the lumbar region, mainly when in the recumbent posture. For some time before this he had had polyuria and nocturnal frequency of micturition ; otherwise his main complaint was of dryness in the mouth and foetor of the breath.

On admission the patient, though a tall man (1·7 metres), weighed only 45 kg., his weight prior to the beginning of the present illness having been 84 kg. Blood-pressure was 140/95. X-ray examination disclosed areas of decalcification in the skull and pelvis, while the long bones appeared normal. A diagnosis of multiple myeloma was first suggested, but later, on a hard nodule being found in the prostate, carcinoma of this organ with bone metastases was thought to be more probable. Treatment with agostilbin was therefore started but brought no relief.

Laboratory data. The urine contained protein on several examinations, but the test for Bence-Jones protein was equivocal. A blood-count on 20th October showed 3·13 million erythrocytes and 10,800 white cells (polymorphonuclears 54, lymphocytes 40, plasma cells 2 and monocytes 2 per cent.) : Hb. 70 per cent. Serum-protein 10·91 per cent. Non-protein nitrogen 162 mg. per 100 c.c. Later there was a progressive rise in the non-protein nitrogen up to 328 mg., and the patient died of uraemia on 2nd November.

Post-mortem examination

This was performed by Dr E. Vaněček 7 hours after death. The following findings were disclosed.

In the *skeletal system* there were numerous soft whitish tumours replacing the bone-marrow and destroying the bony tissue. The seventh rib on the right side showed a circumscribed swelling caused by a mass of white tumour tissue replacing the bone for a length of 2 cm. Smaller tumours of similar aspect were found in the calvarium and in the bone-marrow of the femur, while several vertebræ were diffusely infiltrated with growth.

The *liver*, which was slightly enlarged (1720 g.), contained several greyish nodules the size of millet seeds ; otherwise the parenchyma was rusty brown. The *spleen* (210 g.) was brick-red on section, with indistinct markings. The *kidneys* showed conspicuous lesions. They were markedly swollen, measuring $14 \cdot 5 \times 7 \times 4$ cm. The capsule stripped easily, leaving a smooth grey surface studded with yellowish dots and streaks. On section the cortex was broad and greyish, with irregular markings, but sharply delimited from the medulla, which was bluish red. Tests for amyloid gave a negative result. The pelvis appeared normal. The *prostate* was only slightly enlarged. It contained several whitish elastic nodules but showed no signs of malignant growth. The *lymph-nodes* were of normal size throughout. The remaining findings were unimportant.

Microscopic examination

Bone tumours. Smears made from a tumour in a vertebra at the post-mortem show plasma cells. Sections were made from tumours of rib and femur. The tissue is composed of round, oval and polygonal cells, 10-12 μ in diameter, with markedly basophilic cytoplasm staining bluish gray with hæmatoxylin. The nuclei are relatively large and mostly situated eccentrically ; the chromatin is rather dense but sometimes appears segmented, and occasionally an indefinite spoked-wheel arrangement is noticeable. There are a few nuclei in mitosis and some of the larger cells have two nuclei. A certain number of particularly large cells with irregularly lobulated nuclei are encountered which have a superficial resemblance to Hodgkin giant cells but possess definitely basophilic cytoplasm. Small foci of necrosis consisting of ghost cells are scattered throughout the tumour tissue.

In some of the tumour cells small rhomboid crystals are seen ; in others there are prismatic crystals of larger size, greatly distending the cell, so that the cytoplasm envelopes the crystal like a thin membrane and the nucleus is much elongated. Besides the tumour cells proper there are rather numerous macrophages containing engulfed nuclear debris and granules of iron-positive blood pigment. These cells increase in number towards the periphery of the tumour. In sections stained for fibrin by Weigert's method the intracellular crystals appear a deep violet ; in addition, numerous prismatic and needle-shaped crystals are seen lying extracellularly between the

tumour cells. The other tumours so far examined have the same structure and they too contain intra- and extracellular crystals.

Spleen. The cells of the red pulp are much increased in number, whereas the Malpighian bodies are small and poorly delimited. Many pulp cells have engulfed erythrocytes, and there is a large quantity of iron-positive pigment also present in them. Inside the distended sinuses are many myeloma (plasma) cells, some of them binuclear, but no crystals can be demonstrated.

Liver. The sinusoids are dilated and compress the trabeculæ. The stellate cells are swollen. Some sinusoids are filled with myeloma (plasma) cells arranged in rows. Both epithelial and stellate cells are heavily laden with iron-positive pigment. The Weigert stain fails to disclose crystals, but the Kupffer cells are found to contain small roundish granules.

Myocardium. Nothing particular is seen here except considerable interstitial œdema. The œdema fluid contains finely floccular or fibrillary masses which stain faintly with eosin. No amyloid is demonstrable by special methods.

Prostate gland. Several fibroadenomas of typical structure are present but there is no evidence of malignancy.

Kidney. The general pattern is much distorted by an increase of the interstitial tissue which is rather densely infiltrated by inflammatory cells of all kinds, including polymorphonuclear and eosinophil leucocytes. The majority of the glomeruli, approximately four-fifths of them, offer a most unusual picture. The Bowman's capsules are much distended and deformed by masses of rhombic and prismatic crystals (fig. 1) which also lie between the coils of the glomerular tuft, causing it to appear disrupted. Many renal tubules also are distended and contain similar crystals, which, however, are considerably larger on the average (fig. 2), some attaining a length of more than 400 μ. They can be found in all segments of the nephron from the glomerulus to the ducts of Bellini, although their size gradually decreases distally and, from the loops of Henle onwards, only small whetstone-shaped crystals are present. Most of the crystals are surrounded by giant-cells of fantastic form and their contours sometimes appear irregularly eroded.

The epithelium of the involved tubules is partly lost, partly flattened or detached from the basement membrane. These cells appear to be proliferating, and it is probably from them that the giant-cells have taken origin. There were also numerous giant-cells lying apparently in the interstitium : these, which sometimes rather resemble Langhans' cells, contain conglomerates of small needle-shaped crystals. It is difficult to say whether they are of mesenchymal origin or whether they represent remnants of destroyed tubules. In the epithelium of some of the better-preserved tubules small granules can be demonstrated which stain by Weigert's fibrin method.

The staining properties of the crystals are as follows. All of them,

even the smallest needles, are stained deep violet-blue by Weigert's fibrin method. In Lugol's solution they become brownish but do not stand out distinctly from the surrounding structures. It is doubtful if they show metachromasia with aniline dyes. The larger crystals, but not the small needles, are also stained by Congo red, if differentiation with lithium carbonate has not been pushed too far. The adjacent giant cells also appear distinctly red. Sudan staining gives completely negative results. With polarised light some of the bigger crystals show slight optical activity.

DISCUSSION

The clinical course of this case of plasma-cell myeloma was unusually rapid, the interval between the first manifestation of symptoms and the death of the patient being only 6 months. It may be assumed, however, that the disease had started much earlier, as the lesions in the kidneys were obviously rather advanced.

The crystalline deposits in the kidneys were very extensive, some four-fifths of the nephrons being completely blocked. The massiveness of this particular lesion is duplicated only in Rehsteiner's case (1922-23). The presence of crystals within the Bowman's capsules seems to be unique. Another remarkable feature is the finding of crystals in the cells of the bone tumours ; this has previously been reported by Glaus (1917), Steinmann (1939-40), Apitz (1940) and Brass (1942-43, 1943-44).

The production of para-protein substances in individual cases of myeloma apparently shows great differences both in quality and in quantity. The qualitative differences are indicated by the fact that in some cases para-amyloid is formed, whereas in others crystalline deposits or hyaline droplets make their appearance. The composition of the abnormal serum protein as well as that of the substance secreted by the kidneys is also variable. Quantitative differences may be inferred from the rapidity with which the damage to the kidneys develops. Thus in some cases there is no renal lesion at all, whereas in others the patient dies of uræmia after a relatively short course, sometimes even before tumours have become clinically manifest.

This is consistent with the general notion of neoplastic anaplasia, although the rate of para-protein production need not be paralleled by that of tumour growth. Our case is an example of extremely high production of para-protein, manifesting itself by definite hyper-proteinæmia as well as by the unusually severe renal damage that had developed in a short lapse of time ; and also by the presence of crystals at the site of para-protein production, namely in the tumour cells themselves. In contrast to this, the case of Šikl, described in the preceding paper, shows a very low growth-rate of the tumour cells, which, instead of forming tumours, spread diffusely in the bone-marrow for more than three years. Here, the production of para-
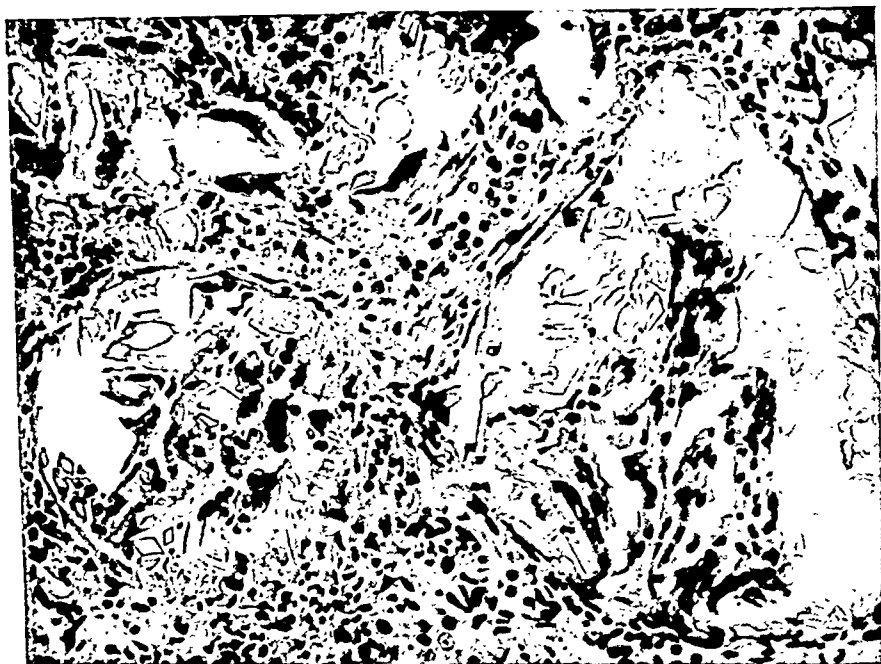
MULTIPLE PLASMA-CELL MYELOMA

FIG. 1.—Crystalline deposits of protein in Bowman's capsules. Hæmatoxylin and eosin. ×190
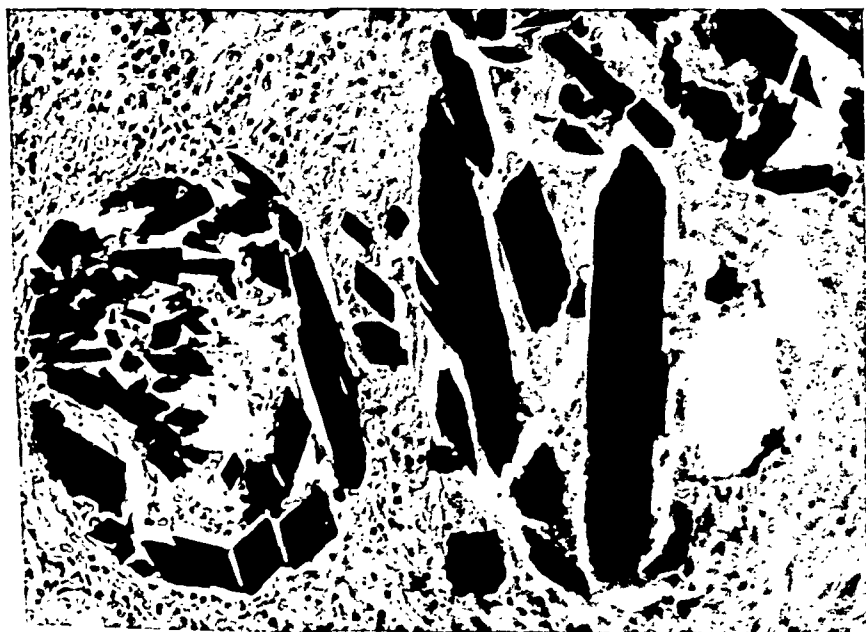

FIG. 2.—Larger crystals in renal tubules, stained by Weigert's method for fibrin. ×240.

protein must also have been much lower, as the kidneys showed only the initial stage of the specific lesion.

Another detail deserving comment is the accumulation of iron-reaction pigment in the spleen and liver. This was obviously the result of hæmolysis, which had also led to definite though not excessive anæmia of hypochromic type. The hæmolysis may be best accounted for by some toxic action of the metabolic products of the tumour cells.

SUMMARY

A case of plasma-cell myeloma is reported in a man of 45, who died after six months' illness. The principal clinical features were great loss of weight, considerable hypochromic anæmia, proteinuria without demonstrable Bence-Jones protein, and X-ray evidence of decalcified areas in the skull and pelvis. Two per cent. of plasma cells were present in the circulating blood. A discrete tumour (plasmocytoma) of the right 7th rib was regarded as the primary lesion. This had given rise to numerous secondary deposits in other bones, especially the skull, vertebral column and long bones, and more diffuse metastatic involvement of the liver and spleen. The kidneys show a most unusual condition. Protein crystals giving a positive reaction with Weigert's fibrin stain and occasionally showing also an affinity for Congo red were present in the Bowman's capsules and nephrons generally in amounts not hitherto recorded. Many of these crystals were of enormous size (up to 400 μ in length). Others of smaller size were present within many of the tumour cells.

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THE EFFECT OF VITAMIN C ON MUCOPOLY-SACCHARIDE PRODUCTION IN WOUND HEALING

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(PLATES XXXV-XXXVII)

HISTOLOGICAL studies of experimental wounds from vitamin C-deficient guinea-pigs have led to the conclusion that impaired healing is associated with failure to form extracellular material (Wolbach, 1933 ; Hunt, 1940-41). Similar results have been obtained in human experiments (Crandon, *et al.*, 1940 ; Hunt, 1940-41 ; Medical Research Council, Accessory Food Factors Committee, 1948).

Wolbach has described in some detail the appearance of sections of wounds from depleted guinea-pigs. He found that, although proliferation and migration of fibroblasts occurred, no extracellular material was formed. However, soon after the administration of vitamin C to the depleted animals a homogeneous amorphous material which stained blue with Mallory's connective tissue stain appeared round the cells. This was followed rapidly by the formation of reticulin fibres embedded in the amorphous material. The production of this material is of interest, since it is the first indication that the repair process has been resumed. The chemical nature of this amorphous material was not established by Wolbach who, for convenience, called it amorphous collagen. Sylvén (1941), studying the formation of granulation tissue in wounds from normal animals, found that substances which stained metachromatically with toluidine blue appeared very early in the repair process. The dye was considered to be specific for mucopolysaccharide sulphuric acid esters. The presence of mucopolysaccharides may be inferred from results of Bensley (1934) on the properties of the material extractable from granulation tissue. In chronic scurvy, Meyer (1943-44) found that the metachromasia of articular cartilage with toluidine blue was greatly reduced, due, he considered, to reduction of the chondroitin sulphate content. The " homogeneous collagen " of Wolbach may, therefore, be " mucopolysaccharide," the formation of which may be dependent on the supply of ascorbic acid.

In this communication we report observations on the chemical nature of the extracellular material produced in the initial stages of normal wound healing and give the results of experiments on the relationship between vitamin C and the formation of this material.

Methods

Growing guinea-pigs of approximately 300 g. weight were maintained on a basal vitamin C-free diet (Penney and Zilva, 1946). For the study of normal

repair, the animals were given either 25 mg. of ascorbic acid daily or cabbage *ad lib.* in addition to the basal diet. Muscle wounds were made as described by Wolbach ; after given intervals the animals were killed by stunning and bleeding and the wound area taken for histological study. Twenty-three normal guinea-pigs were killed at daily intervals from the 4th to the 9th day after operation. The remaining animals were wounded after 13 days on the vitamin C-free diet and were either killed at daily intervals from the 5th to the 9th day after operation (17 animals), or injected intramuscularly with 25 mg. of ascorbic acid on the 7th day after wounding and killed 6, 12, 18, 24 or 48 hours later (11 animals). Immediately after death the wound area was excised, fixed in 10 per cent. neutral formalin for 24 hours, in Helly's fluid or in 80 per cent. alcohol and embedded in paraffin.

For general histological studies the stains used were hæmatoxylin and van Gieson, Foot's modification of Bielschowsky's silver impregnation method, Weigert's iron hæmatoxylin and Unna's methyl-green pyronin.

For the demonstration of acid mucopolysaccharides, the metachromatic staining properties of toluidine blue were utilised, following, usually, the procedure described by Sylvén. We have found that there is a considerable variation between different batches of the dye. Some samples gave satisfactory metachromatic staining after ordinary alcohol dehydration. With other samples the metachromasia, although visible when examined under water, was destroyed by alcohol, but in some cases could be preserved by using dioxane as the dehydrating agent. This variation in the behaviour of the stain may account for the different staining techniques reported in the literature. In this investigation every batch of dye was tested on control sections taken from the same block and only those which gave good metachromatic staining after alcohol or dioxane dehydration were used.

Although the metachromasia with toluidine blue was originally believed to be specific for mucopolysaccharide sulphuric acid esters (Lison, 1936), it was later reported that this effect is also produced by nucleoproteins (Wislocki *et al.*, 1947) and hyaluronic acid in high concentration (Meyer, 1947). We therefore attempted to increase the specificity of the method by observing the effect of hyaluronidase and ribonuclease on the substances which exhibit metachromasia. In the first instance de-paraffinised sections were treated with purified ox-testis hyaluronidase in one per cent. saline (activity 2000 M.C.U./ml.) or with one per cent. saline alone for four hours at room temperature (20°-22° C.). Sections from the same blocks were incubated with purified ribonuclease (10 mg./100 ml.) at *p*H 6·7 or with buffer solution alone for 1 hour at 37° C. After treatment, all the sections were stained with toluidine blue and compared with the untreated sections.

Results

Normal group. In this group fibroblastic and vascular invasion of the clot was observed 4 days after wounding. By the 5th day several stages of the repair process could be seen in sections from the same wound. At the periphery of the wound, elongated and orientated fibroblasts were found associated with van Gieson-staining fibres. Fibroblasts which had advanced further into the clot were triangular or rhomboidal and were usually in the form of a syncytium. Stearns (1940) has reported that this arrangement precedes fibre formation. Associated with the fibroblasts were both fine and coarse reticulin fibres. After staining with toluidine blue, metachromasia was evident in the cytoplasm of the fibroblasts and in the extracellular material.

Vitamin C and Wound Healing

FIG. 1.—Normal granulation tissue, showing fibrous metachromatic extracellular material and cytoplasm of fibroblasts exhibiting metachromasia. Toluidine blue. ×290.

FIG. 3.—Scorbutic "granulation tissue," showing absence of metachromatic extracellular material and abnormal cell appearance. Toluidine blue. ×290.

FIG. 6.—Granulation tissue from initially vitamin C-depleted animal following ascorbic acid administration, showing fine metachromatic fibres embedded in a pale metachromatic background. Twelve hours after ascorbic acid administration. Toluidine blue. ×700.

FIG. 10.—Normal granulation tissue. Toluidine blue. ×70.

FIG. 11.—Normal granulation tissue after treatment with purified testis hyaluronidase. Toluidine blue. ×70.

VITAMIN C AND WOUND HEALING
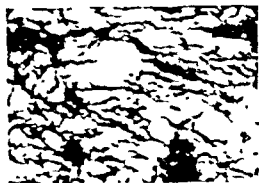
FIG. 2.—Normal granulation tissue,
showing the argyrophil fibres
usually associated with meta-
chromasia. Foot's modification
of Bielschowsky's silver stain.
×290.

FIG. 7.—Serial section to fig. 6, show-
ing argyrophil fibres corresponding
to metachromatic fibres. Foot's
modification of Bielschowsky's silver
stain. ×700.
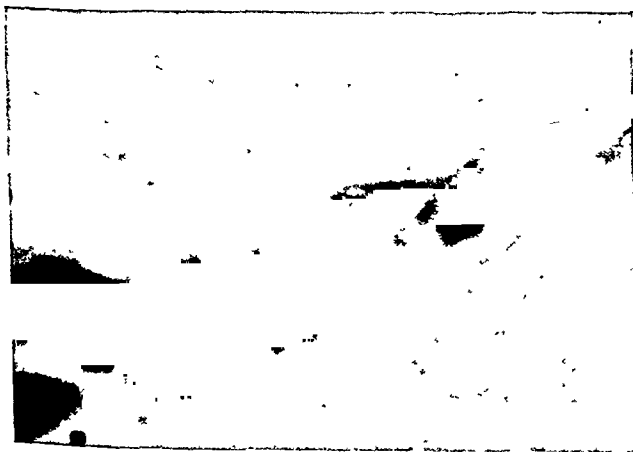
FIG. 4.—Abnormal vessel
from scorbutic wound,
showing absence of
lumen. Weigert's iron
hæmatoxylin. ×1890.

FIG. 5.—Abnormal vascular
proliferation in scorbutic
wound, showing heaping
up of cells around patent
vessel. Weigert's iron
hæmatoxylin. ×1890.

A close correlation was noted between the distribution of newly formed fibres and the extracellular metachromasia. In areas where the fibroblasts had migrated deeply into the clot, the metachromatic staining material was sometimes found in amorphous form with very fine argyrophil fibres embedded in it. Usually, however, it had a fibrous structure, resembling the pattern of both the fine and coarser reticulin fibres (figs. 1 and 2), suggesting that the argyrophil fibres themselves possessed metachromatic staining properties. Towards the periphery of the wound area, the coarser bundles of toned and van Gieson-staining fibres were clearly metachromatic. On increasing the post-operative period to 9 days, more van Gieson-staining fibres were observed. As these fibres mature, they lose their metachromatic staining properties (*cf.* Sylvén).

In addition to the metachromasia associated with fibroblasts, areas of amorphous material which gave the characteristic metachromatic staining could be found far removed from the cells in the depth of the wound.

Depleted group. When sections of wounds from this group taken five days after operation were examined, it was found that some fibroblastic proliferation had occurred. The cells had migrated into the clot, but as noted by Wolbach they were very abnormal in form and distribution. They were pleomorphic and rarely attained the characteristic appearance of normal fibroblasts. Many cells had small densely staining nuclei and scanty amounts of cytoplasm, while in others a hyperchromatic nuclear membrane surrounded an almost clear nucleus with no nucleolus. Cells with large vacuoles, generally found near those engaged in phagocytosis, are probably histiocytes; in some areas these were the predominating cell types. Very small vacuoles were sometimes observed in the abnormal fibroblasts; these appeared to correspond to small fat droplets found in frozen sections.

The distribution of the cells in the wound area differed greatly from that found in normal wounds. The fibroblasts were generally separated and never formed a syncytium except in small areas near the periphery of the wound. In these areas the cell form closely resembled that of a normal fibroblast.

Although the cells had proliferated and migrated into the clot, very little extracellular material had been formed (fig. 3). Since, on increasing the post-operative period up to 9 days, no further attempts at repair appeared to be made, the following remarks refer to sections taken over the whole period studied.

Small areas of reticulin fibres associated with metachromasia were sometimes observed at the periphery of the wound, where the fibroblasts appeared less abnormal. It is of interest that occasionally these areas were in close proximity to the frayed and disintegrating ends of pre-formed collagen fibres. In this condition the collagen exhibited metachromasia and became argyrophil. As the fibroblasts penetrated further into the clot, however, no attempt at fibre formation

was observed and no metachromatic staining material associated with cells could be found, either with the formol- or Helly-fixed material. Amorphous material which stained metachromatically was sometimes observed in the clot away from fibroblasts, resembling, in this respect, the normal wounds.

Metachromasia of the cytoplasm was not a constant finding. Those cells which were almost normal in appearance had this property, but the abnormal cells with scanty cytoplasm varied in their staining reactions.

Limited capillary proliferation was also a characteristic of depleted wounds and the new-formed capillaries were often abnormal. They frequently failed to form a lumen and corresponded to the closed columns of endothelial cells described by Wolbach (fig. 4). In addition, heaping up of the cells around patent vessels could be observed (fig. 5). Differentiation of the vessels, obvious in the normal process of repair, did not take place. It is clear from these observations that the blood supply to the more remote parts of the wound area is inadequate and the abnormal appearance of some of the cells in these areas may be attributable to inanition.

After the intramuscular administration of ascorbic acid to depleted animals on the 7th day of the post-operative period (20th day of complete depletion), striking differences in staining properties and cell morphology were observed. A large increase in the amount of metachromatic staining material was found after 12 hours, and even after 6 hours there was evidence of increased production of these substances. By 12 hours it was noted that some isolated cells which had penetrated into the clot were surrounded by confined regions of amorphous material showing metachromasia. Very fine reticulin fibres were distributed through some but not all of these areas. In regions containing considerable numbers of cells much extracellular material had been produced and fine reticulin fibres associated with metachromasia were much in evidence (figs. 6 and 7). On increasing the interval after ascorbic acid administration, fibre formation took place rapidly and by 24 hours van Gieson-staining and toned fibres had appeared.

Vigorous cellular activity was also noted in the perivascular connective tissue surrounding the larger arteries. Here it appeared that the cells were concerned with newly formed fibrous extracellular material which stained metachromatically but was not argyrophil.

There was a rapid change in the morphology of the cells accompanying the formation of extracellular material. In 12 hours many of the cells had attained the appearance of normal fibroblasts. Both cytoplasm and nuclei had enlarged and the latter had become vesicular, with prominent nucleoli. After this interval the cytoplasm of the normal and of some of the abnormal cells was metachromatic. It should also be noted that some of the cells which had not yet
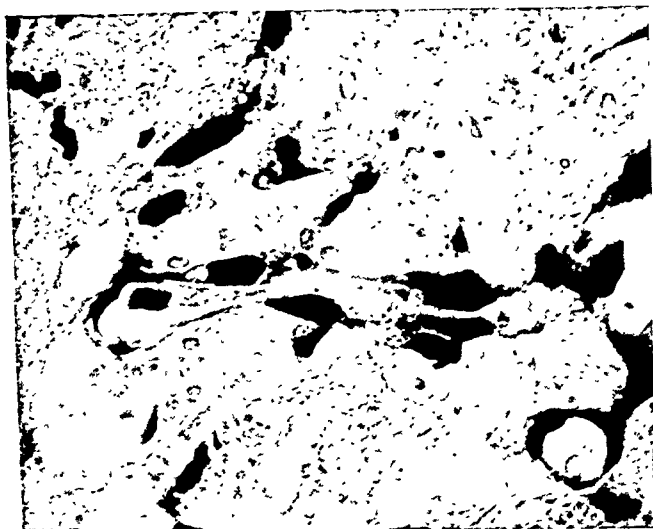
VITAMIN C AND WOUND HEALING

FIG. 8.—Vascular proliferation following ascorbic acid administration to initially vitamin C-depleted animal. Formation of lumen in non-patent vessel. Weigert's iron hæmatoxylin. × 700.
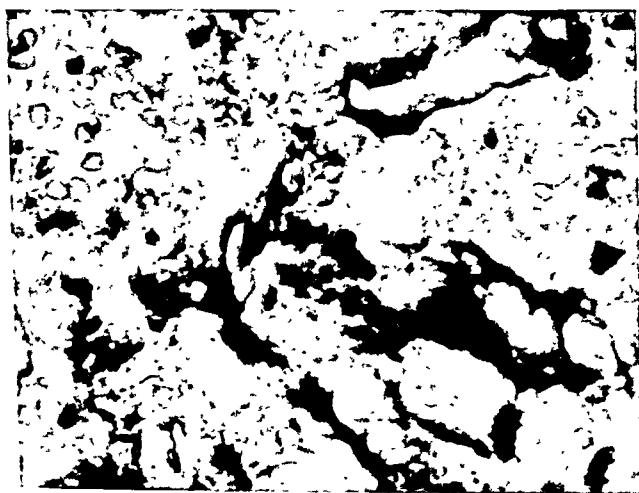

FIG. 9.—Vascular proliferation following ascorbic acid administration to initially vitamin C-depleted animal, showing perivascular reticulin sheath appearing 18 hours after ascorbic acid administration. Foot's modification of Bielschowsky's silver stain. × 783.

attained the appearance of normal fibroblasts were associated with newly formed extracellular material. Forty-eight hours after ascorbic acid administration all the cells were normal in appearance.

The development of capillaries also responded rapidly to ascorbic acid therapy. During the experimental period, non-patent vessels developed a lumen ; this change was more noticeable at the periphery of the wound (fig. 8). In areas containing newly formed reticulin fibres it was sometimes found that fine fibres had become attached to the vessel wall. Where a vessel had penetrated into the clot unaccompanied by fibroblasts, argyrophil fibres formed a definite perivascular sheath (fig. 9).

Enzyme treatment of sections

In these experiments, sections were taken of 7-day wounds from control and depleted animals and from injected animals. After treatment with hyaluronidase, or 1 per cent. saline, the sections were stained with toluidine blue and compared with untreated sections. One per cent. saline did not affect the resulting metachromasia. Hyaluronidase, however, removed the substances responsible for the metachromasia in the newly formed extracellular material (figs. 10 and 11) leaving a non-metachromatic residue. This provides good evidence that these substances are mucopolysaccharides of the hyaluronic acid or chondroitin sulphate type. The metachromatic material of the cytoplasm did not appear to be affected by hyaluronidase.

We should like to record that the metachromatic staining of disintegrating collagen, of amorphous material in the depths of the wounds, of the media of larger blood vessels and of the perineurium was abolished by hyaluronidase, whereas the granules of mast cells were unaffected.

After treatment with ribonuclease no metachromasia could be observed in the cytoplasm, but the extracellular material was unaffected. Selective removal of the metachromasia from the cytoplasm of the endothelial cells sometimes revealed very fine metachromatic staining fibres outlining capillaries. This could be seen more easily in the wounds taken from the injected animals 12 hours after ascorbic acid administration.

Discussion

The appearance of substances which stain metachromatically in granulation tissue, described in some detail by Sylvén, has been confirmed. On the basis of this staining property he suggested that both the cytoplasmic and extracellular metachromasia were due to the presence of mucopolysaccharide sulphuric acid esters. Our observations using toluidine blue in conjunction with testicular hyaluronidase indicate that the extracellular substance or substances giving rise to metachromasia are acid mucopolysaccharides but we do not feel justified in attempting to characterise these substances

more definitely at this stage. The possibility of hyaluronic acid giving metachromasia cannot be excluded (Stacey, 1946; Meyer, 1947; Wislocki *et al.*, 1947), although it should be remembered that we took no special precautions to retain it (*cf.* Leach, 1947). Furthermore, the enzyme used by us is known to degrade both hyaluronic acid and chondroitin sulphate (Humphrey, 1946). The use of the specific streptococcus hyaluronidase might help to elucidate this point.

The metachromasia of the fibroblasts is probably due to the presence of ribo-nucleoproteins, since it can be removed by ribonuclease. This is in keeping with the high cytoplasmic nucleic acid content found in growing and protein-secreting cells.

The failure of wound repair in depleted animals is associated with the failure of formation of extracellular material in both homogeneous and fibrous form. It is of interest that the stage at which the repair process is retarded depends on whether the supply of ascorbic acid is completely withheld or whether sub-minimal amounts are supplied. Hence Danielli *et al.* (1945) found that on low doses of ascorbic acid (less than 2 mg. per day) large amounts of reticulin could be formed although the wound appearance was not normal. In preliminary experiments we have obtained indications that on these low doses of ascorbic acid large amounts of mucopolysaccharide are formed.

The appearance of mucopolysaccharide and reticulin after the injection of ascorbic acid to depleted animals was very rapid, and was accompanied by a change in cell morphology. Since mucopolysaccharide was found associated with fibroblasts which were still abnormal, we are inclined to the view that resumption of the reparative process precedes the attainment of normal cell morphology. Within 12 hours of ascorbic acid administration considerable amounts of mucopolysaccharide were found, and although areas of homogeneous material were sometimes found alone, the mucopolysaccharide was very often associated with fine reticulin fibres. Wolbach was able to demonstrate more clearly the production of a homogeneous extracellular material using aniline blue, and by virtue of its staining properties termed the material " amorphous collagen." The distribution of this material appears to correspond very closely to that of the mucopolysaccharides found in our experiments. It is probable that by the injection of large doses of ascorbic acid the response in our experiments was more rapid and the successive stages not so clearly defined as in Wolbach's experiments, in which the animals were given orange juice. Similarly the difficulty in observing well-defined stages in normal healing is probably due to the rapidity of the process.

From the evidence available it would appear that the first event leading to fibre formation is the deposition of mucopolysaccharide about the fibroblasts in homogeneous form. These substances are known to occur in nature in association with protein and we have observed a non-metachromatic-staining residue in sections after

treatment with hyaluronidase. This residue might be responsible for the staining results obtained by Wolbach with aniline blue. We do not, however, consider that the available evidence is sufficient to indicate that a true homogeneous protein precursor of collagen is present, as suggested by Meyer (1947). The initial production of mucopolysaccharide is followed by the formation of very fine argyrophil fibres embedded in the homogeneous material. Thickening of these fibres is accompanied by a change in the distribution of the muco-polysaccharide, which now takes a fibrous form corresponding to the argyrophil fibres. This suggests that the fibre protein is closely connected with the mucopolysaccharide, either in physical combination (Meyer, 1947) or as a complex. After maturation of the fibres, the mucopolysaccharide can no longer be demonstrated histologically. The mechanism of fibre formation is not yet understood, although the in-vitro production of fibres can be brought about by acidification of a solution containing gelatin and chondroitin sulphate (Meyer *et al.*, 1937). Whether a similar mechanism is involved in the in-vivo production of fibres is obscure.

The possibility of mucopolysaccharide disturbance in scurvy has been reported by Meyer (1943-44), who found that the metachromasia of articular cartilage was very much reduced in the chronic form of this disease. Such a disturbance might also account for the hæmorrhages associated with the deficiency. Recently Chambers and Zweifach (1947) have obtained evidence for the presence of mucopolysaccharide in the connective tissue sheath of the blood capillaries. Any failure in mucopolysaccharide formation might result in the weakening of the sheath, leading to hæmorrhages. It is of interest that the connective tissue sheath associated with proliferating vessels can be demonstrated histologically by its meta-chromatic properties.

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The metachromasia of the fibroblasts is probably due to the presence of ribo-nucleoproteins, since it can be removed by ribonuclease. This is in keeping with the high cytoplasmic nucleic acid content found in growing and protein-secreting cells.

The failure of wound repair in depleted animals is associated with the failure of formation of extracellular material in both homogeneous and fibrous form. It is of interest that the stage at which the repair process is retarded depends on whether the supply of ascorbic acid is completely withheld or whether sub-minimal amounts are supplied. Hence Danielli *et al.* (1945) found that on low doses of ascorbic acid (less than 2 mg. per day) large amounts of reticulin could be formed although the wound appearance was not normal. In preliminary experiments we have obtained indications that on these low doses of ascorbic acid large amounts of mucopolysaccharide are formed.

The appearance of mucopolysaccharide and reticulin after the injection of ascorbic acid to depleted animals was very rapid, and was accompanied by a change in cell morphology. Since mucopolysaccharide was found associated with fibroblasts which were still abnormal, we are inclined to the view that resumption of the reparative process precedes the attainment of normal cell morphology. Within 12 hours of ascorbic acid administration considerable amounts of mucopolysaccharide were found, and although areas of homogeneous material were sometimes found alone, the mucopolysaccharide was very often associated with fine reticulin fibres. Wolbach was able to demonstrate more clearly the production of a homogeneous extracellular material using aniline blue, and by virtue of its staining properties termed the material " amorphous collagen." The distribution of this material appears to correspond very closely to that of the mucopolysaccharides found in our experiments. It is probable that by the injection of large doses of ascorbic acid the response in our experiments was more rapid and the successive stages not so clearly defined as in Wolbach's experiments, in which the animals were given orange juice. Similarly the difficulty in observing well-defined stages in normal healing is probably due to the rapidity of the process.

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SITES OF ANTIBODY PRODUCTION

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IN a skilfully planned and conclusive set of experiments McMaster and Hudack (1935) showed that if suspensions of killed typhoid bacilli or of sheep red cells are injected into the ears of mice, antibody is produced earliest and in highest concentration in the lymph glands draining the injection area, and that the antibody is produced whether the injection site is subsequently excised or not. Later McMaster and Kidd (1937) extended these experiments to vaccinia virus, and concluded that antivirus antibodies, like those to bacterial vaccines and sheep red cells, are produced to a large extent in the lymph glands draining the area injected with antigen. Burnet and Lush (1938) and Burnet *et al.* (1941) obtained similar results for influenza and for herpes virus. Ehrich and Harris (1942) injected the feet of rabbits with killed typhoid bacilli and sheep red cells ; cannulation of the afferent and efferent lymphatics of the popliteal lymph glands (through which, in the rabbit, the whole lymph of the foot flows) showed that antibody is produced in the lymph gland and carried largely by lymphocytes.

In the experiments referred to above, as in most of the published experiments on the site of antibody production, the antigens used were killed bacterial suspensions or sheep red cells, no doubt because antibodies to them are produced rapidly and are readily titrated *in vitro*. The methods for the detection of such antibodies are of high sensitivity but low precision. As some antitoxins can be tested, either *in vivo* or *in vitro*, at low concentrations with much higher precision, an attempt has been made in this paper to extend the work of McMaster and Hudack (1935) and of Ehrich and his collaborators (Ehrich and Harris, 1942) by using the response to the injection of bacterial toxoids. The secondary type of antitoxin response to second or subsequent injections of antigen (Glenny and Südmersen, 1921) has been studied throughout.

EXPERIMENTAL

1. *Secondary response to the injection of a single antigen*

Guinea-pigs, rabbits or horses were primarily stimulated with a single injection of diphtheria alum-precipitated toxoid (5 c.c. intramuscularly in the neck in horses ; 0·2 c.c. subcutaneously in the neck or abdominal wall in

rabbits or guinea-pigs). At some time over a month later, the same amount of the antigen was injected into that part of the right hind leg draining into the popliteal gland, and the animal bled out at some convenient time thereafter. Both popliteal glands were collected and ground separately with powdered quartz and saline, the volume was made up to a suitable figure, the mixture centrifuged, and the diphtheria antitoxin content of the supernatant estimated by the intracutaneous method of Römer and Sames (1909) as worked out by Glenny and Llewellyn-Jones (1931).

In all cases the total amount of diphtheria antitoxin and the amount per gramme of gland recovered from the popliteal lymph gland draining the injected limb was greater than in the gland draining the uninjected limb (table I). Many of the published claims for

TABLE I

Comparison of antitoxin recovered from lymph glands draining injected and uninjected limbs in animals injected with diphtheria alum-precipitated toxoid

Animals	Units of diphtheria antitoxin in gland draining			
	injected limb		uninjected limb	
	Total	Units/g.	Total	Units/g.
Rabbits . .	0·010	0·059	<0·0025	<0·023
	0·065	0·26	0·0035	0·024
	0·006	0·043	<0·0025	<0·027
Guinea-pigs .	0·335	5·5	0·025	0·483
	0·035	2·75	<0·005	<0·34
	0·063	2·06	0·0035	0·168
Horses . .	424·0	36·8	146·0	2·27.
	17·3	2·16	0·22	0·1
	17·8	3·24	6·36	0·676

antibody production in various organs are based on little more evidence than this ; but the claim that relatively greater recovery of antibody from an organ is evidence of local production is essentially fallacious. All the animals used by us had circulating diphtheria antitoxin when they were killed ; in every instance the lymph gland draining the injection area was larger than that on the other side and markedly inflamed ; antibody might readily have leaked into the inflamed gland from the circulation. McMaster and Hudack (1935) and Walsh and Cannon (1938) dealt admirably with this difficulty. Perhaps more important, taking these experiments at their face value assumes that our methods of extracting antibody are uniformly efficient. Since this cannot be guaranteed, the experiments were modified to avoid this difficulty by injecting two or more antigens in different sites in the experimental animals and determining the antitoxin response to each. However inefficient the method of extraction of a

tissue, it will presumably be equally efficient for all antitoxins present. If the tissues are inflamed, antitoxins will leak into them from the circulation in the proportions in which they occur in the plasma and will be extracted in those proportions. Deviations of the antitoxin ratios of tissues from those in the plasma may then be regarded as evidence of production or storage of one or other antitoxin in those tissues.

2. *Secondary response to injections of two antigens*

Horses used for the commercial production of lamb dysentery or pulpy kidney serum, and producing, therefore, a variety of *Clostridium welchii* antitoxins, were given 5 c.c. of diphtheria alum-precipitated toxoid (A.P.T.) and 10 c.c. of tetanus A.P.T. intramuscularly into the neck; the injections were repeated one month later. The horses were subsequently given 5 c.c. of diphtheria A.P.T. injected subcutaneously into the right hind leg below the knee, and 10 c.c. of tetanus A.P.T. injected similarly into the left hind leg. At convenient times thereafter horses were bled out, and the popliteal, femoral, axillary and mesenteric lymph glands, and pieces of heart, liver, spleen, kidney, lung, leg injection areas and neck injection areas were collected, freed from fat as far as possible, and the tissue extracts and serum examined for antitoxins. Diphtheria antitoxin was estimated by intracutaneous testing in guinea-pigs; tetanus antitoxin by subcutaneous injection of tetanus toxin-extract mixtures into mice; *Cl. welchii* β antitoxin by intracutaneous testing of β toxin-extract mixtures in guinea-pigs; *Cl. welchii* ϵ antitoxin by intracutaneous testing of ϵ toxin-extract mixtures in rabbits; *Cl. welchii* α antitoxin by in-vitro hæmolytic tests of α toxin-extract mixtures, using washed sheep red cells as indicator.

Thirty horses have so far been used; results for two of them, typical of the whole group, are given in tables II and III. It is clear that the tetanus/diphtheria antitoxin ratio in the lymph glands draining the leg injected with diphtheria A.P.T. is lower than the ratio of these antitoxins in the serum; for the lymph glands draining the leg injected with tetanus A.P.T. the tetanus/diphtheria antitoxin ratio is higher than it is in the serum. The simplest explanation for this is that the lymph glands draining the injection area have made or stored relatively more antitoxin to the antigen with which their drainage area has been injected.

The possibility that some other antitoxin may be produced or stored in the lymph glands draining an area injected with a particular antigen may also be tested. Horses immunised for the commercial production of lamb dysentery or pulpy kidney serum are injected intramuscularly in the neck with *Cl. welchii* type B filtrates (chiefly α, β and ϵ toxins) or with *Cl. welchii* type D filtrates (chiefly α and ϵ toxins). Estimation of antibody to α, β or ϵ toxins in tissues from such horses would be expected to yield information on the amount of antitoxin derived from the circulation, either by leakage or from failure to bleed out completely. If the ratio of two antibodies in a tissue is the same as the ratio in the circulation, the simplest explanation is that both antibodies are derived from the circulation. Tables IV

and V show the ratios for diphtheria, tetanus and α or ϵ antitoxins of tissues from two pulpy kidney horses injected with diphtheria

TABLE II

Comparison of tetanus/diphtheria antitoxin ratios in extracts of lymph glands draining right and left legs of horse 5790, injected with diphtheria A.P.T. (right hind leg) and tetanus A.P.T. (left hind leg), and bled out 18 days later

Material tested	Antitoxin units per ml.		Tetanus/diphtheria antitoxin ratio
	Diphtheria	Tetanus	
Extract of left popliteal lymph gland	0·0017	0·5	290·0
Extract of left femoral lymph gland	0·004	0·7	180·0
Extract of right popliteal lymph gland	0·056	0·15	2·7
Extract of right femoral lymph gland	0·02	0·2	10·0
Serum　.　.　.　.	0·34	14·5	43·0

Ratios in this and subsequent tables are given to 2 significant figures.

TABLE III

Comparison of tetanus/diphtheria antitoxin ratios in extracts of lymph glands draining right and left hind legs of horse 5555, injected with diphtheria A.P.T. (right hind leg) and tetanus A.P.T. (left hind leg), and bled out 7 days later

Material tested	Antitoxin units per ml.		Tetanus/diphtheria antitoxin ratio
	Diphtheria	Tetanus	
Extract of left popliteal lymph gland	1·8	38·0	21·0
Extract of left femoral lymph gland	1·5	30·0	20·0
Extract of right popliteal lymph gland	21·0	1·5	0·071
Extract of right femoral lymph gland	25·0	3·0	0·12
Serum　.　.　.　.	40·0	80·0	2·0

A.P.T. (right hind leg) and tetanus A.P.T. (left hind leg). It is clear from these results that the ratio of, for example, *Cl. welchii* α or ϵ antitoxin to diphtheria antitoxin in the lymph glands draining the limb injected with tetanus A.P.T. is not significantly different from that in the circulation, and that the same applies to the *Cl. welchii* α or ϵ/tetanus antitoxin ratio in the lymph glands draining the limb injected with diphtheria A.P.T. In other words if a limb is injected with diphtheria or tetanus A.P.T., there is no evidence of corresponding antibody production or storage in the lymph glands draining the opposite limb. Similar arguments can be applied to antibody ratios

for other tissues. As table VI shows, the ϵ/diphtheria/tetanus antitoxin ratios for many organs are not significantly different from those for

TABLE IV

Comparison of antitoxin ratios in extracts of lymph glands draining left and right hind legs of horse 5790, injected with diphtheria A.P.T. (right hind leg), tetanus A.P.T. (left hind leg) and Cl. welchii α toxin (neck), and bled out 18 days later

Material tested	Antitoxin ratios		
	Tetanus/diphtheria	Cl. welchii α/diphtheria	Cl. welchii α/tetanus
Extract of left popliteal lymph gland	290 0	130	0·45
Extract of left femoral lymph gland	180 0	180	1·0
Extract of right popliteal lymph gland	2·7	15	5·5
Extract of right femoral lymph gland	10 0	38	3·8
Serum	43 0	120	2·8

TABLE V

Comparison of antitoxin ratios in extracts of lymph glands draining left and right hind limbs of horse 5555, injected with diphtheria A.P.T. (right hind leg) tetanus A.P.T. (left hind leg) and Cl. welchii ε toxin (neck), and bled out 7 days later

Material tested	Antitoxin ratios		
	Tetanus/diphtheria	Cl. welchii ε/diphtheria	Cl. welchii ε/tetanus
Extract of left popliteal lymph gland	21·0	7·7	0·36
Extract of left femoral lymph gland	20 0	6·7	0·33
Extract of right popliteal lymph gland	0 071	0·30	4·2
Extract of right femoral lymph gland	0 12	0 40	3·3
Serum	2 0	7 2	3 6

the serum ; no evidence of differential production or storage could be found.

It is tempting to regard these experiments as demonstrating not only that antibody to diphtheria and tetanus alum-precipitated toxoid is produced or stored in the lymph glands draining the injection area, but also that no other structure so far examined (liver, kidney, spleen, muscle, heart, lung, mesenteric gland) produces any antibody at all. The latter conclusion can, however, be challenged from two points of view.

First, the errors of antitoxin testing in this experiment must be considered : for our diphtheria antitoxin determinations about

TABLE VI

Comparison of antitoxin ratios in extracts of various structures of horse 6102, injected with diphtheria A.P.T. (right hind leg) tetanus A.P.T. (left hind leg) and Cl. welchii ε toxin (neck) and bled out 8 days after

Material tested	Antitoxin ratios		
	Tetanus/diphtheria	Cl. welchii ε/diphtheria	Cl. welchii ε/tetanus
Extract of left popliteal lymph gland	150·0	...	...
Extract of left femoral lymph gland	290·0	180·0	0·62
Extract of right popliteal lymph gland	2·1	13·0	6·0
Extract of right femoral lymph gland	0·97	9·7	10·0
Extract of mesenteric gland	38·0	210·0	5·7
„ heart . .	27·0	380·0	14·0
„ liver . .	27·0	160·0	6·0
„ spleen . .	20·0	140·0	7·0
„ kidney . .	24·0	210·0	9·1
Extract of left injection area (leg)	32·0	360·0	11·0
Extract of right injection area (leg)	33·0	300·0	9·0
Extract of left axillary lymph gland	13·5 *	...	...
Extract of right axillary lymph gland	28·0	300·0	11·0
Serum	21·0	180·0	8·7

* This low value may be due to the injection of diphtheria A.P.T. into the neck several months previously.

± 5 per cent. for results above 0·1 unit, ± 10 per cent. for lower values ; for our tetanus, α and ε antitoxin determinations ±10 per cent. ; for our β antitoxin titrations ±15 per cent. ; the errors of the ratios will certainly be greater than those of their components. We have therefore taken little note of differences in antitoxin ratios as evidence of antitoxin production unless the differences were very large, and we may have missed something thereby. For instance, if a large organ like the liver produced only 10 per cent. of the total diphtheria antitoxin it contained, the rest being derived from the circulation, the total amount produced might be very large but we should be unable to show it. The same applies to other organs, whose production —on a 10 per cent. basis—might be greater than that of the draining lymph gland.

Second, it may fairly be argued that if antigens reach an organ from a distance, the relative concentrations of antigens reaching the organs may be constant and the organ responses correspondingly uniform ; the antibody ratios in the organs might then be equal to

those in the blood. This may be true, and we propose to test its truth by finding whether organs will rapidly produce antitoxin differentially when injected with diphtheria and tetanus A.P.T. in different places. If they do not, we may reasonably assume that they produce no antitoxin when the antigens reach them from a distance.

In this connection the perfusion experiments of Sédallian *et al.* (1939*a, b*) are worth noting. They secondarily stimulated rabbits with diphtheria or tetanus toxoid ; a few days later they removed chosen organs or structures from these rabbits and established continuity between the vessels of the isolated structures and the circulations of similarly immunised rabbits whose serum antitoxin levels had reached a steady state. If antitoxin had been produced in sufficient quantity in the isolated structures, the serum antitoxin of the perfusing rabbit would have risen. In fact no rise in serum antitoxin occurred in rabbits perfusing isolated liver or kidney ; well-marked increases in serum antitoxin occurred in rabbits perfusing the isolated hind legs, into which the dose of antigen used as a secondary stimulus had been injected. Sédallian *et al.* (1939*b*) concluded that the antitoxin came from the bone marrow, and indeed it is difficult to see how it could have come from the popliteal lymph glands in their experiments, unless there is free communication between lymph glands and veins in the leg.

It may be worth noting here that, in horses injected with A.P.T. in the legs, the antitoxin ratios in the femoral glands were usually nearer to those of the serum (tables III and IV) than were the ratios of the popliteal glands. Presumably the lymph reaching the femoral from the popliteal glands is diluted with lymph of similar antitoxin ratio to that in the circulation. Unfortunately, as we do not know the relative proportions of popliteal and other lymph draining into the femoral glands, we cannot decide whether any antitoxin is produced in the femoral glands ; but the small difference in antitoxin ratio between popliteal and femoral glands makes it seem likely that the femoral glands produce some antitoxin.

So far we have not provided any evidence to distinguish between production and storage in the draining lymph gland. Ehrich and Harris, by cannulating the afferent and efferent lymphatics of the popliteal gland of the injected limb of rabbits, found little antibody in the afferent lymphatics and much more in the efferent, which strongly suggested antibody production in the lymph gland. Our evidence is less direct. In most cases the injection areas in the hind limbs of horses receiving diphtheria and tetanus A.P.T. in the usual way show tetanus/diphtheria antitoxin ratios equal to those in the serum (table VII) ; similar results are found for the muscle and connective tissue lying between the skin and the draining gland (table VIII). In these horses we may be reasonably confident that antitoxin has been produced only in the draining lymph gland. But

in a few horses, *e.g.* 5330 and 5408, and sometimes only on one side, the ratio of homologous to heterologous antibody in the injected skin

TABLE VII

Tetanus/diphtheria antitoxin ratios in extracts of injection areas from 5 horses injected with diphtheria A.P.T. (right hind leg) and tetanus A.P.T. (left hind leg), to show evidence of tetanus antitoxin production in the skin injection area of horses 5330 and 5408

Material	Tetanus/diphtheria antitoxin ratio for horse				
	5793	5848	6102	5330	5408
Serum	0·85	33	21	240	7·3
Extract of left leg injection area .	0·68	25	32	6400	56·0
Extract of right leg injection area .	0·69	23	33	170	4·9
Days after last injection . .	6	5	8	5	10

TABLE VIII

Tetanus/diphtheria antitoxin ratios for extracts of various structures of two horses injected with diphtheria A.P.T. (right hind leg) and tetanus A.P.T. (left hind leg). There is no evidence of antibody production or storage except in the lymph glands. Note evidence of differential antibody production 29 days after injection of antigen

Material tested	Tetanus/diphtheria antitoxin ratio for	
	horse 5912	horse 9469
Extract of left popliteal lymph gland . .	30·0	22·0
„ left femoral lymph gland . .	40·0	21·0
„ left leg muscle	24·0	3·2
„ left thigh muscle . . .	15·0	3·4
„ right leg muscle	18·0	3·3
„ right thigh muscle . . .	18·0	3·8
„ right popliteal lymph gland . .	1·9	…
„ right femoral lymph gland . .	3·1	1·2
Serum	18·0	3·3
Days after last injection	15	29

is much higher than the ratio for the same antibodies in the serum (table VII). Antitoxin production has evidently occurred in the skin. Further evidence on this point was obtained from rabbits.

3. *Local production of antibody in skin*

Rabbits were given primary injections of 0·2 ml. of diphtheria A.P.T. into the dorsum of the right hind foot, and 0·2 ml. tetanus A.P.T. into the dorsum of the left hind foot. One month and two months later the injections were repeated, diphtheria A.P.T. into the right foot, tetanus A.P.T. into the left foot. In order to increase the amount of bone-marrow the rabbits were bled

about one-third of their estimated blood volume ten days before they were killed. At various times after the third injection the rabbits were bled out from the carotid, and the injection areas, the glands draining them and the femoral and tibial bone marrow were excised and their extracts tested for diphtheria and tetanus antitoxins.

Table IX shows that in all cases the skin from the areas injected with tetanus A.P.T. and the glands draining them showed a higher

TABLE IX

Tetanus/diphtheria antitoxin ratio for extracts of structures and serum from 6 rabbits, injected with diphtheria A.P.T. twice at an interval of a month in right foot, and twice similarly with tetanus A.P.T. in left foot, to show evidence of antibody production in the injection area

Material tested	Tetanus/diphtheria antitoxin ratio for rabbit					
	1	2	3	4	5	6
Serum	11·0	5·5	3·3	1·7	5·0	1·0
Extract of left bone marrow	14·0	6·7	3·1	2·2	4·5	1·7
Extract of right bone marrow	7·7	6·0	3·5	2·0	5·1	1·6
Extract of left foot injection area	55·0	56·0	97·0	45·0	200 0	100·0
Extract of right foot injection area	0·54	0·3	0 073	<0·6	<0·15	<0·11
Extract of left popliteal gland	48·0	38·0	42·0	14·0	3·3	3·6
Extract of right popliteal gland	0 64	0·35	<0·022	0·38	0·93	<1·2
Days after last injection .	5	5	7	7	10	10

tetanus/diphtheria antitoxin ratio than the serum; on the side injected with diphtheria A.P.T. the skin and draining glands showed a tetanus/diphtheria antitoxin ratio less than that of the serum. The bone-marrow extracts in the main showed ratios equal to those in the serum. Antitoxin had evidently been produced in the injected skin.

Some light was thrown on the difference between some horses and rabbits by a consideration of the different lesions produced on injection. As already stated, the primary injections in the horses were made into the muscles of the neck and the second injections in the skin of the hind limbs were made into normal tissue. Since the animal was usually bled out less than ten days later, the reaction was diffuse and had little time to localise itself. In the rabbit, at least two injections were made in the same area, the last at a time when the granuloma that normally follows the injection of A.P.T. had had time to form there. Possibly antitoxin produced in rabbit skin is derived from the cells of the pre-formed granuloma. This possibility will be tested by injecting diphtheria A.P.T. once into the right foot and twice into the left foot, and tetanus A.P.T. once into the right ear

and twice into the left ear of rabbits primarily stimulated in the neck or abdominal wall. If the view here put forward is correct, in an experiment of this kind, skin with two injections at an interval of a month or so should show antitoxin production about five days after the second injection ; skin with one injection should show no antitoxin production five days later.

4. *Continuance of antitoxin production*

Barr and Glenny (1947) showed that horses under immunisation continue to produce antibody long after the injection of antigen is stopped, and that they settle down to a steady rate of production after a fall in their antitoxin level which continues for a variable time depending on the length of the immunisation. The longer a horse has been under continuous immunisation, the sooner it reaches a steady level, and the higher the ratio its steady antitoxin level bears to its antitoxin titre at the time immunisation is stopped.

Our experiments have not been carried on long enough for our information to be comparable with that given by Barr and Glenny ; but it is clear from table X that local differential production of antitoxin may go on for as long as eight months after a secondary injection.

TABLE X

Tetanus/diphtheria antitoxin ratios of serum and of extracts of various structures from horses receiving either one injection in the neck and one in the leg, or two injections in the leg, of diphtheria A.P.T. (right hind leg) and tetanus A.P.T. (left hind leg) to show evidence of antibody production in lymph gland (7727) and in injection area (6338, 6368) long after the last stimulus

Material tested	Tetanus/diphtheria antitoxin ratio for horse		
	6368	6338	7727
Extract of left popliteal lymph gland	64	...	23·0
Extract of left femoral lymph gland	...	...	50·0
Extract of right popliteal lymph gland	18	...	7·0
Extract of right femoral lymph gland	...	...	7·7
Extract of left leg injection area .	91	>760	14·0
„ right leg injection area	18	...	14·0
Serum	...	130	15·4
Number of injections in leg . .	2	2	1
Days after last injection . .	66	129	271

DISCUSSION

The literature on the organs concerned in antibody production shows clearly that extensive removal of organs may have little effect

on antibody response, *e.g.* Buttle (1934). Hektoen and Curtis (1915) showed that splenectomy produced a slight delay in production and a lower final concentration of antibody to rat red cells injected intravenously into dogs : removal of the small intestine and ligation of the mesenteric artery delayed the appearance of antibody, a result attributed by Hektoen and Curtis to the large amount of lymphoid tissue removed with the intestine. It is not very clear whether the great natural variation in response to antigens was seriously considered in these experiments. In ablation experiments Harris *et al.* (1948) found no evidence that the thymus produced or stored antibody to *Shigella dysenteriæ*, *Salmonella typhi* or sheep erythrocytes injected subcutaneously or intravenously. If the antigen was injected subcutaneously the spleen played no obvious part in producing or storing antibody ; if the antigen was injected intravenously, the concentration of antibody in the spleen was higher than in the other tissues : this the authors regard as evidence of localised antibody production (*cf.* Pfeiffer and Marx (1898) for lysins against *Vibrio choleræ* in rabbits : in table III of their paper the < and > signs appear to be reversed).

The great difficulty in producing much effect on antibody production by ablation of organs has led authors to ascribe antibody production to generally distributed structures such as lymph glands, plasma cells and reticulo-endothelial tissue. Here the literature displays a regrettable narrowness of outlook, in that authors support the claims of macrophages, lymphocytes or plasma cells with scant recognition of the claims of their opponents.

Jaffé's (1931) review of the vast literature on the reticulo-endothelial system and immunity shows that almost all the evidence in favour of its participation in antibody production is indirect and of doubtful validity ; the original papers often supply evidence that no account has been taken of the leakage of antibody from the circulation into inflamed tissue. The extensive literature on reticulo-endothelial blockade shows that small doses of blocking substance may stimulate antibody production, large doses depress it (Roberts, 1929*a*, *b* ; Cannon *et al.*, 1929).

Ehrich and his collaborators have given excellent evidence for the importance of the lymphocyte (Ehrich and Harris, 1942 ; Harris *et al.*, 1945), and Ehrich *et al.* (1946) state that polymorphs and macrophages from antibody-producing areas contain no antibody (*cf.* Pfeiffer and Marx). As the supporters of the reticulo-endothelial origin of antibodies (*e.g.* Sabin, 1939) have always pointed out the appositeness of antibody production by cells that have ingested and digested the antigen, it is interesting to note that Harris and Ehrich (1946) have claimed that killed bacterial suspensions and sheep red cells are altered at the site of injection, possibly by macrophages, to substances that act as blocking antigens. Thus if killed typhoid bacilli are injected into the rabbit's foot, extracts from the injection area 24 hours later contain no recognisable bacilli, but prevent agglutination of

typhoid bacilli by specific agglutinating sera. Similar substances can be recovered from the popliteal gland, but disappear as antibody is produced there. It is therefore concluded that these altered antigens stimulate antibody production by lymphocytes in the draining lymph gland.

The case for the lymphocytes receives a good deal of support from experiments in which the lymphoid tissue is severely damaged by X-rays. Thus Hektoen (1915, 1918) found that large doses of X-rays applied to white rats, dogs and rabbits in doses sufficient to damage the lymphoid tissue would, if given daily for some time before the antigen was injected, delay the antibody response to intraperitoneal injection of sheep red cells, or even abolish it completely. At the height of the antibody response neither X-ray treatment nor splenectomy, nor both together, had much effect on antibody levels in the serum (Hektoen, 1920). Murphy and Sturm (1925) confirmed Hektoen's observations with doses that produced severe damage to lymphoid tissue but did not injure the bone-marrow, and extended them to the production of precipitins, bacterial agglutinins and protective antibodies : they also showed that dry heat, which stimulates lymphoid tissue, increased antibody production. Evans (1948) found that patients irradiated with X-rays for the treatment of malignant disease and those whose lymph glands were extensively infiltrated with leukæmic masses showed a poor response to killed " H " suspensions of *Bact. paratyphosum* A as compared with normal controls.

As it is generally agreed that antibodies resemble normal globulins, the claim that lymphocytes produce antibodies led to an examination of their power to produce globulin, and it was soon shown (White and Dougherty, 1946) that lymphocytes contain a substance electrophoretically similar to γ globulin ; the suggestion has thus been made that lymphocytes normally synthesise γ globulin. Dougherty *et al.* (1945) also showed that other substances damaging lymphoid tissue —adrenal cortical extract and pituitary adrenotropic hormone—release antibody into the circulation of animals whose antibody level has fallen below a detectable limit. As cortical extract was effective in producing this effect in adrenalectomised animals, they claimed that the pituitary stimulates the cortex of the adrenal to produce a hormone which causes lymphocytes to break up and release antibody ; in their view this explains the anamnestic reaction. On the other side it should be stated that Andreasen *et al.* (1948) found no fall in plasma protein in rats from which they had removed surgically at least 90 per cent. of the lymphoid tissue, and Harris and Henle (1948) found no evidence of an anamnestic reaction in rabbits in which a severe lymphopenia had been induced by the intravenous injection of influenza virus. Likewise Philips *et al.* (1947) found that poisoning goats with nitrogen mustards, which damage myelopoietic and lymphopoietic tissues, produced only some delay in the secondary response to ricin, without reducing the maximum serum antibody

level reached. Murphy and Sturm (1947) found that adrenalectomised animals had an enhanced capacity to produce antibody.

Recently the claims of the plasma cell were taken up by Bjørneboe and Gormsen (1943), who drew attention to the marked plasma-cell infiltration of the spleen and liver in animals injected with large amounts of soluble antigens, *e.g.* pneumococcal polysaccharides. Injections of india ink and globulin, to produce the hyperglobulinæmia common in immunised animals, did not lead to plasma-cell infiltration. Bjørneboe *et al.* (1947) also drew attention to the high concentration of plasma cells and antibody in the peri-renal fat in their immunised animals.

This work was ably extended by Fagraeus (1946, 1947, 1948), who claimed that after intravenous injection of horse serum or live or killed *Salm. typhi* into rabbits the antigen was localised mainly in the red pulp of the spleen, both absolute and relative rates of destruction of *Salm. typhi* being greater in the red pulp than in the follicles. Soon the reticulum cells of the pulp began to develop into plasma cells; and if pieces of splenic pulp were grown in tissue culture, increase of antibody in the cultured fragments ran parallel to the concentration of immature plasma cells in them. The most marked difference in antibody concentration between splenic pulp and serum was observed about four days after a secondary stimulus; after seven days the difference was of doubtful significance. It would be useful to extend these experiments to other organs.

Our experiments throw little light on these controversies, though our methods could readily be adapted to semi-microdissection techniques like those of Fagraeus. We did attempt to estimate antitoxin ratios in the cortex and medulla of lymph glands, imagining that if macrophages actively produce antitoxin, the medulla would show an antibody ratio higher than that of the cortex; in fact the differences were such as to suggest that antitoxin in passing into the medulla is diluted with lymph having the same antitoxin ratios as the plasma.

On the question of local antibody production we need not say much, as the evidence has been thoroughly reviewed in Burnet's remarkable monograph (Burnet *et al.*, 1941). It is, however, necessary to refer to Walsh and Cannon, who seem to have been the first to demonstrate local antibody production (in the respiratory tract) by the injection of two different antigens by different routes, and the estimation of antibodies to both in various tissues. From their paper it is possible to show that if one antigen (A) is instilled into the nose and the other (B) injected intraperitoneally, the anti-A/anti-B ratio in the nasal mucosa is higher than that in the circulation, whereas the anti-A/anti-B ratio in the spleen is lower than in the circulation. Burnet *et al.* injected four different antigens into four different sites in the skin of rabbits but found no evidence of local antibody production, possibly because the experiment did not last long enough

for a granuloma to form. It seems to us fair to conclude that antibody may be produced in a variety of places and probably by a variety of cells, depending on the antigen and the route by which it is injected.

Numerous interesting problems arise from work done so far. If antitoxin is produced mainly in the lymph glands draining a particular area, higher antitoxin levels might be expected from multiple injections of antigen in different areas, so that many lymph glands might respond. If it is the filtering capacity of lymph glands that determines the local production of antibody, we might also reasonably expect a better response to a diffusible antigen, *e.g.* toxoid, which might reach numerous structures. Burnet *et al.* found little evidence of local antitoxin production in rabbits injected with staphylococcus toxoid.

It is truly remarkable that a primary stimulating dose of a particular antigen, draining presumably through only a few lymphatic glands, alters other lymph glands in such a way that they produce antibody in amounts characteristic of a secondary response when subjected to a secondary stimulus. Possibly the claim of Sjövall (1936) that lymphocytes circulate between lymph gland, circulation and lymph gland may help to explain this. Unfortumately the antitoxin level reached in a primary response is usually so low that organ extracts from the animal cannot be tested.

Much more work will be necessary to throw light on these problems.

SUMMARY

After secondary stimulation of horses, rabbits and guinea-pigs by the subcutaneous injection of diphtheria or tetanus alum-precipitated toxoid, antitoxin is produced in the lymph glands draining the injected area. In some cases antibody may be produced in the injected skin, especially if the area so injected has already been primarily or secondarily stimulated with the same A.P.T., with production of a local granuloma.

No evidence has been obtained of antibody production elsewhere, though the possibility cannot be excluded ; if it occurs, as much as 90 per cent. of the total antibody might be produced outside the injection area and its draining lymphatic glands without our methods being able to demonstrate it.

Differential local production of antitoxin may occur up to 271 days after the injection of diphtheria or tetanus alum-precipitated toxoid.

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THE CYTOCHEMICAL DEMONSTRATION OF GONADOTROPIC HORMONE IN THE HUMAN ANTERIOR HYPOPHYSIS

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(PLATES XXXVIII AND XXXIX)

THE problem of deciding which particular cell is responsible for which of the battery of pituitary hormones described by various workers has hitherto been approached by two different yet essentially similar methods : (1) by correlating changes in the relative number of the three types of cell, acidophil, basiphil and chromophobe, with anatomical or physiological changes in the body of the animal concerned ; (2) by a cytological study of these cells in various natural and experimental conditions, on the assumption that an increase in size and number of the specific granules in the chromophils with a parallel increase in the size of the Golgi apparatus indicates an increased secretory effort on the part of the cells concerned.

The first method has been used in the determination of the universally accepted relationship between the acidophil cells and the growth hormone in cases of acidophil adenoma and gigantism or acromegaly. Additional evidence in support of this relationship is found in the occurrence of dwarfism in mice and men in cases showing total or almost total absence of the acidophils (Smith and MacDowell, 1930 ; Hewer, 1942-44).

In the case of the gonadotropic hormones a combination of both methods has been used, and it is concluded that the increased amount of gonadotropin recoverable from the urine of castrated animals is to be related to the increase in number and activity of the basiphils which accompanies it. This increased production has also been demonstrated by assay of excised hypophyses for their hormone content (Engle, 1929).

In her very comprehensive review of the available evidence, Severinghaus (1937) proposes that this fits in best with a scheme which assumes that the basiphils produce the follicle-*stimulating* hormone (F.S.H.) while the acidophils are responsible for the luteinising hormone (L.H.). These methods are presumptive only and a cyto-chemical test should give a more direct answer.

The chemistry of the gonadotropins

According to Meyer's (1938) classification, naturally-occurring hexosamine-containing compounds fall into three groups :—(A) neutral and acid mucopolysaccharides (which include all the true mucins); (B) mucoproteins ; and (C) glycoproteins.

In the last two classes, hexose and hexosamine are linked to protein to form an integral part of the molecule and an arbitrary division between the two is fixed at a hexosamine content of less than 4 per cent. for the glycoproteins. The gonadotropic hormones have been proved to be proteins containing both hexose and hexosamine in their molecule. As regards the follicle-stimulating fraction from the sheep's anterior hypophysis, Evans *et al.* (1939) have shown that it has a hexose content of 10·3 to 13·1 per cent. and 8 per cent. of glucosamine. It is therefore a mucoprotein. These authors state that the luteinising fraction contains from 3·6 to 5·4 per cent. of mannose and 3·8 to 5·8 per cent. of hexosamine, and Gurin (1942) has shown that human chorionic gonadotropin contains 10-12 per cent. of galactose and 5-6 per cent. of hexosamine. These, therefore, are also mucoproteins. The purest fraction of ox-pituitary growth hormone, on the contrary, contains only 0·25 per cent. of sugar and less than 0·9 per cent. of glucosamine (Fraenkel-Conrat *et al.*, 1940) and is therefore neither a mucoprotein nor a glycoprotein. Figures for the sheep are a little above this level.

None of these hormones has as yet been obtained in an absolutely pure form, but the striking difference between the various growth-hormone fractions on the one hand and the gonadotropins on the other in respect of their carbohydrate and hexosamine content is sufficiently well established.

The conception of applying the cytochemical technique described below to the problem of localising the source of the gonadotropins is based, therefore, on the assumption that human follicle-stimulating and luteinising hormones are probably both mucoproteins or glyco-proteins and that their cell source or sources might be revealed by a method capable of demonstrating these substances.

The periodic-acid method in histology

The use of periodic acid (HIO_4) in histology was described by McManus (1946) for the demonstration of mucin and independently by Hotchkiss (1948), who elaborated the method into a histochemical one with adequate controls. McManus (1948a) once regarded the method as primarily of histological usefulness but (1948b) now accepts Hotchkiss's view that it can be used, properly, as a histochemical test.

Periodic acid is an oxidising agent sufficiently strong to break the C-C bond in monosaccharides and it oxidises the 1.2. glycol grouping (CHOH-CHOH) to a di-aldehyde. The equivalent amino or alkyl-

DIFFERENTIAL STAINING OF PITUITARY CELLS

FIG. 1.—Basiphils and chromophobes of pituitary gland for comparison with fig. 2. Gram's stain. ×540.
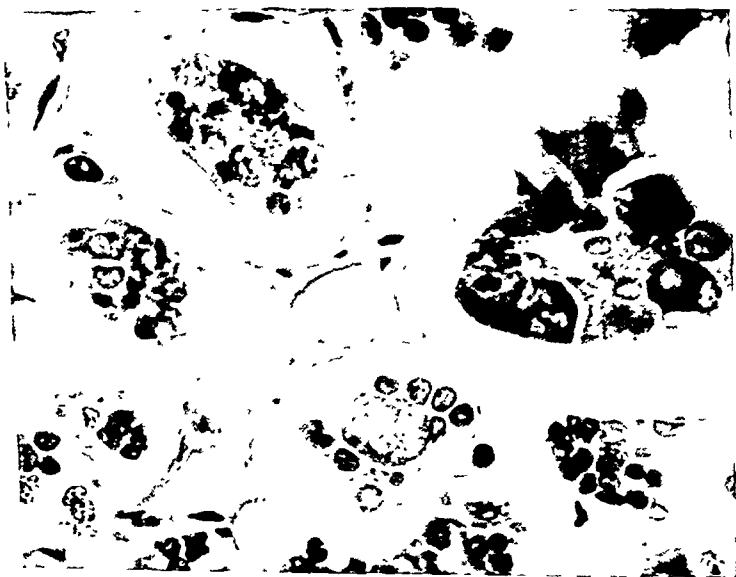
FIG. 2.—Serial section to fig. 1, showing Schiff-positive basiphils and Schiff-positive vesicles in the chromophobes. Periodic acid-Schiff, celestin blue, hæmalum. ×540.

PLATE XXXIX

DIFFERENTIAL STAINING OF PITUITARY CELLS
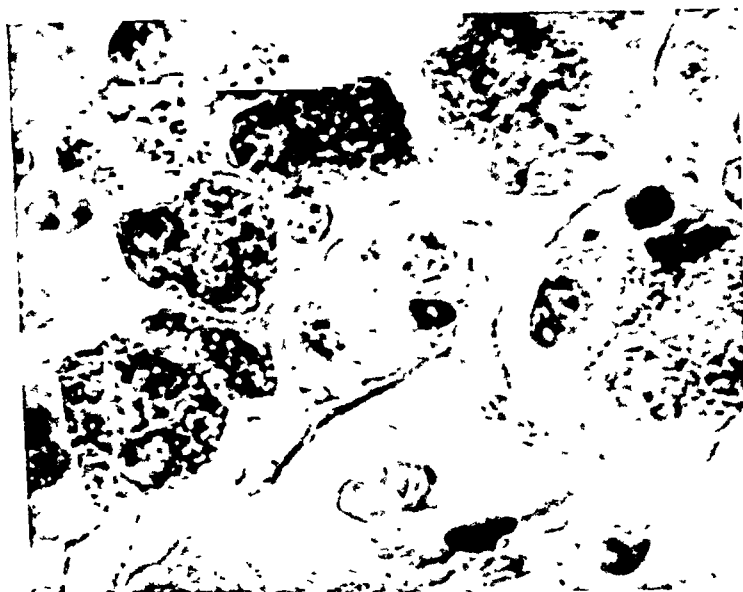
FIG. 3.—Schiff-positive β granules in the basiphil cells. Three acidophils occupy the centre of the field. Periodic acid-Schiff, celestin blue, hæmalum. ×1100.
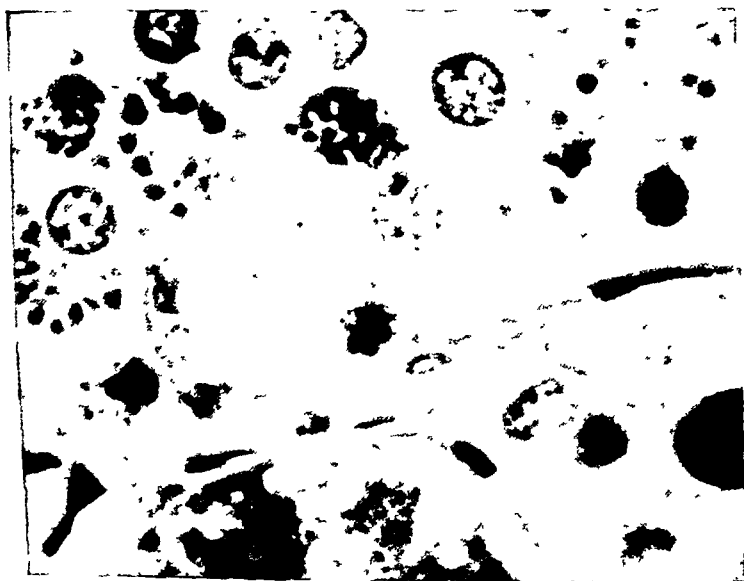
FIG. 4.—*Lower right*, positive staining secretion in an acinus: *lower centre*, β granules in basiphils: *upper left*, vesiculate chromophobes: the two large pale cells in the centre are acidophils. Periodic acid-Schiff, celestin blue, hæmalum. ×1100.

amino derivatives of 1.2. glycol or its oxidation product CHOH-CO are also attacked and converted to aldehydes. These, however, are not oxidised to any extent by periodic acid and can be combined with leucofuchsin (Schiff's reagent) to give a red dye.

Theoretically a large number of substances contain the 1.2. glycol grouping for which the reaction is specific and should therefore give positive results but, according to Hotchkiss, naturally-occurring animal substances which in fact do so are monosaccharides, polysaccharides, mucoproteins, glycoproteins, phosphorylated sugars, cerebrosides and inositol-containing lipoids. After the use of ordinary aqueous fixatives only substances of high molecular weight should remain in the tissues in sufficient quantity to give a positive reaction. These substances are mucopolysaccharides, mucoproteins and glycoproteins.

In fact, large amounts of the important polysaccharide glycogen and small amounts of sugar-containing lipoids also remain. Glycogen may be removed by short hydrolysis with ptyalin or diastase, while Hotchkiss endeavours to remove the cerebrosides and other lipoids by using 70 per cent. alcoholic solutions. As Lison (1936) points out, fixation renders lipoids histologically insoluble, though their chemical characteristics indicate ready solubility, and these lipoids also remain to complicate the procedure.

In practice, however, bearing in mind these objections, a positive result with a negative control indicates mucopolysaccharide, mucoprotein or glycoprotein. The amount of the leuco- dye converted into the red substituted dye is dependent on the weight of glycol structure present in the tissue concerned and this is highest in the first group and lowest in the third. Substances containing less than 1-2 per cent. of hexose or hexosamine give only a faintly positive reaction.

Application of the method

The cytochemical part of the method is applicable after any of the usual fixatives, and the cytological part is improved by those which contain mercury or dichromate. Sections are treated with a solution of periodic acid in 70 per cent. alcohol, this solvent being chosen on theoretical grounds, since mucoproteins and their oxidation products are soluble in water and might be dissolved away by aqueous solutions during the reaction. Excess of periodic acid, including any which has combined with metals present in the tissues, is removed by the use of an acid reducing rinse. Schiff's reagent is then applied to the sections and also to untreated controls.

Mucoproteins are stained red in the treated sections only. In order to make the sections comparable the nuclei are then stained with celestin blue and the α granules with orange G. A modification of Berblinger and Burgdorf's (1934-35) phosphotungstic acid—orange G has been found most effective. The method finally adopted is as follows, in which stages 3 and 5 follow exactly the technique of Hotchkiss.

Technical details

1. Treat with iodine and thiosulphate to remove salts of mercury.
2. Bring sections to 70 per cent. alcohol.

3. Leave for 5 minutes in periodic acid solution A (*vide infra*) at room temperature.

4. Flood with 70 per cent. alcohol, transfer to reducing rinse B and leave for 1 minute.

5. Flood with 70 per cent. alcohol, leave for 15-45 minutes in fuchsin-sulphite solution C.

6. Wash in running water for 10-30 minutes. In routine work the sulphite baths of Feulgen's method are unnecessary.

7. *Stain the nuclei in 0·5 per cent. celestin blue in 5 per cent. iron alum* (Lendrum and McFarlane, 1940) *for 30 seconds and Mayer's hæmalum for 30 seconds or longer.* If sections have previously been treated with ribonuclease the times in this stage may be increased without limit.

8. Differentiate quickly in 2 per cent. acid-alcohol and blue in water. If desired, dehydration, clearing and mounting may be done at this stage.

The acidophil granules are stained as follows :—

9. Stain in 2 per cent. orange G in 5 per cent. phosphotungstic acid (solution D) for 5-10 seconds.

10. Wash in running water until a yellow tinge is just visible in the acidophil areas, or control under the microscope.

11. Bring through the alcohols to xylol and mount in D.P.X.

Staining reactions

" Colloid " of stalk and parenchyma and the vesicles
of the vesiculate chromophobes .   .   .   .   Magenta
Basiphil granules   .   .   .   .   .   .   Dark red
Acidophil granules   .   .   .   .   .   .   Orange
R.B.C.   .   .   .   .   Orange
Nuclei   .   .   .   .   Blue-black

Solutions required

A. 400 mg. periodic acid (HIO$_4$) are dissolved in 10 ml. of distilled water with 5 ml. of a $M/5$ sodium acetate buffer (27·2 g./litre of the hydrated salt) and 35 ml. of ethyl alcohol.

B. One gramme of potassium iodide and one gramme of sodium thiosulphate pentahydrate are dissolved in 20 ml. of distilled water with 30 ml. of ethyl alcohol and 0·5 ml. of $2N$ HCl. The precipitate of sulphur which forms is ignored.

C. Schiff's solution of Feulgen (Lison, p. 178) or any of its modifications may be employed.

D. Two per cent. orange G (C.I. no. 27) in 5 per cent. aqueous phosphotungstic acid. Orange II (C.I. no. 151) may be substituted for orange G with little change in the quality of the staining. The colour is deeper and more resistant to removal from the background structures.

RESULTS

Applying this method to human hypophyses removed routinely at autopsy, fixed in Helly, Zenker, half-saturated mercuric chloride in formol-saline or formol alone, it is found that the " colloid " of the stalk, the " colloid " in the parenchyma of the gland (fig. 4) and the β granules of the basiphils are all stained positively. The α granules are completely colourless after periodic acid-Schiff (P.A.S.) and take the orange counterstain. Examination of material, in serial sections, by

P.A.S. and by Gram's and Mallory's methods leaves no doubt that it is the β granules which are Schiff-positive, no trace of red being found in the acidophil granules (figs. 3 and 4).   Furthermore the absolute identity of the basiphils with the P.A.S.-positive cells may be established by staining with P.A.S., identifying the cells, removing the red dye by bleaching with acid permanganate and re-staining the basiphil material in the same cells with celestin blue or other basic dye.   The granules of the basiphils retain their affinity for the basic dyes after conversion of their carbohydrate by periodic acid and its fixation by leuco-fuchsin, part of this being due to the strongly dissociated acid groups of nucleoprotein which remain intact.   This nucleoprotein (ribonucleic acid) is removable by hydrolysis with ribonuclease, as shown by Desclin (1940), and the true β granules which remain are no longer strongly basiphil.   They are still P.A.S.-positive but are now less basiphil than the α granules, staining only faintly after 24 hours in 5×10 M methylene blue at pH 6·4, whereas the α granules are deep blue at this pH.

The true β granules are thus P.A.S.-positive and only weakly basiphil in neutral solutions.   They do not show the strong metachromasia characteristic of mucopolysaccharides when stained with dilute aqueous thionin or toluidine blue and they are stable to both diastase and hyaluronidase.   It is therefore concluded that they consist of muco- or glycoprotein.

These findings are directly opposed to those of McManus (1948b), who now states that " some of the acidophile cells of the human hypophysis " stain positively, though his previous references (1946, 1948a) were only to " certain pituitary cells."   They agree with those of Catchpole (1947), who says that a glycoprotein material is associated with the granules of castrate cells in rats.

In a variable proportion of chromophobes, predominantly those in the basiphil areas of the gland, are found accumulations of small vesicles of varying size which stain bright magenta-red (figs. 2 and 4). These can be seen in unstained preparations as isotropic refractile bodies and the colour produced in them by the periodic acid-Schiff method resembles that of the " colloid " very closely, though it differs appreciably from the dark red of the basiphil granules.   Some chromophobes contain two or three vesicles only, while others are filled with them.   They also occur in many of the basiphil cells and in these the number of β granules is often reduced.   In 130 pituitary glands, ranging from a 10-weeks' foetus to a woman of 84, 14 were found with Schiff-positive vesicles in cells with orange acidophil cytoplasm, but true vesicles do not seem to occur in cells containing σ granules.   These sometimes show a fine P.A.S.-positive dusting in the Golgi region of the cell, which is possibly connected with this apparatus.

The nature of the vesicles in the vesiculate chromophobes cannot be decided with certainty, but the possibility of their being lipoidal

3. Leave for 5 minutes in periodic acid solution A (*vide infra*) at room temperature.

4. Flood with 70 per cent. alcohol, transfer to reducing rinse B and leave for 1 minute.

5. Flood with 70 per cent. alcohol, leave for 15-45 minutes in fuchsin-sulphite solution C.

6. Wash in running water for 10-30 minutes. In routine work the sulphite baths of Feulgen's method are unnecessary.

7. Stain the nuclei in 0·5 per cent. celestin blue in 5 per cent. iron alum (Lendrum and McFarlane, 1940) for 30 seconds and Mayer's hæmalum for 30 seconds or longer. If sections have previously been treated with ribonuclease the times in this stage may be increased without limit.

8. Differentiate quickly in 2 per cent. acid-alcohol and blue in water. If desired, dehydration, clearing and mounting may be done at this stage.

The acidophil granules are stained as follows :—

9. Stain in 2 per cent. orange G in 5 per cent. phosphotungstic acid (solution D) for 5-10 seconds.

10. Wash in running water until a yellow tinge is just visible in the acidophil areas, or control under the microscope.

11. Bring through the alcohols to xylol and mount in D.P.X.

Staining reactions

" Colloid " of stalk and parenchyma and the vesicles of the vesiculate chromophobes	Magenta
Basiphil granules	Dark red
Acidophil granules	Orange
R.B.C.	Orange
Nuclei	Blue-black

Solutions required

A. 400 mg. periodic acid (HIO_4) are dissolved in 10 ml. of distilled water with 5 ml. of a $M/5$ sodium acetate buffer (27·2 g./litre of the hydrated salt) and 35 ml. of ethyl alcohol.

B. One gramme of potassium iodide and one gramme of sodium thiosulphate pentahydrate are dissolved in 20 ml. of distilled water with 30 ml. of ethyl alcohol and 0·5 ml. of $2N$ HCl. The precipitate of sulphur which forms is ignored.

C. Schiff's solution of Feulgen (Lison, p. 178) or any of its modifications may be employed.

D. Two per cent. orange G (C.I. no. 27) in 5 per cent. aqueous phosphotungstic acid. Orange II (C.I. no. 151) may be substituted for orange G with little change in the quality of the staining. The colour is deeper and more resistant to removal from the background structures.

RESULTS

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The nature of the vesicles in the vesiculate chromophobes cannot be decided with certainty, but the possibility of their being lipoid

or composed of lipoid-coated mucoprotein has been considered.   They are not doubly refracting and cannot be stained with osmic acid or with Scharlach R, but they can be demonstrated with neutral red or Sudan black B in paraffin sections.   Neutral red, however, colours the colloid and the basiphil granules as well.

From these results the following conclusions are drawn :—

1. The granular material in the basiphils is a mucoprotein and represents the gonadotropic (follicle-stimulating) hormone, with or without luteinising hormone or its precursor.   The vacuoles in normal basiphils do not contain the hormone.

2. The acidophils, because of the complete absence of red colouration in the α granules, secrete neither follicle-stimulating nor luteinising hormone.   This is in contrast to the views of Severinghaus mentioned above.

3. The material in the acini in all parts of the gland contains gonadotropin.   This may well be the reason for discrepancies shown by methods employing bio-assay of presumed acidophil and basiphil portions of the hypophysis (Smelser, 1944).

(4) The vesiculate chromophobes are likely to represent a pre- or post-mature stage in the secretory cycle of the basiphil.   Their apparent increase in post-menopausal females and in elderly males, in whom the secretion of follicle-stimulating hormone is normally raised to a high level, with their relative absence from prepubertal hypophyses, supports this contention.

Serial counts of the three types of cell in the manner of Rasmussen (1929, 1933) have not yet been done.

With the new method, results are clearer than with Mallory's stain and differentiation of the cell types is easier.   It is apparent from preliminary counts of small selected fields that there are two major discrepancies between Mallory's and the present method :—(1) A variable proportion of the aniline blue chromophobes contain the positive-staining vesicles and might better be classified as basiphilic chromophobes.   (2) Other Mallory chromophobes contain frank β granules and obviously belong to the basiphil series.

OTHER SITES IN WHICH MUCOPROTEIN IS FOUND

Periodic acid-Schiff positive material in the form of granular masses, globules and vesicles is found to occur in the trophoblast layer of the placenta and in the Langhans cells of chorionepithelioma. The granular part of this material is removed by diastase and therefore consists of glycogen, but the globules and vesicles are saliva-fast and probably of mucoprotein nature : they may thus represent the chorionic gonadotropin.

Two substances additional to those listed by McManus (1948*a* and *b*) which stain positively are the granules of connective tissue mast cells and the acidophil globules, known as Russell bodies, which are found

in the plasma cells of the lamina propria of the alimentary tract and in various granulomata. The former are believed to contain heparin, an acid mucopolysaccharide which should stain strongly by the periodic acid-Schiff method, while the Russell bodies are brightly positive and probably contain mucoprotein rather than mucopolysaccharide. They are the subject of a short paper published elsewhere (Pearse, 1949).

Summary

1. In the sheep and ox the pituitary gonadotropic hormones are mucoproteins, as are the chorionic gonadotropins in man and animals.

2. The periodic acid-Schiff method can be used as a histochemical test for mucoproteins, *inter alia*, and by its use the gonadotropic hormone in the cells of the hypophysis can be localised.

3. The hormone is found to be present in the basiphils and in the " colloid " of both stalk and parenchyma.

4. A particular type of vesiculate chromophobe has been described and it is suggested that this may represent a phase in the secretory cycle of the basiphil.

5. Positive-staining material is found in the human and animal placenta and in the cells of chorionepithelioma ; this may represent the chorionic gonadotropin.

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   E. C.

ON THE MODE OF FORMATION OF LAMBL'S EXCRESCENCES AND THEIR RELATION TO CHRONIC THICKENING OF THE MITRAL VALVE

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(PLATES XL-XLIV)

LAMBL'S EXCRESCENCES

IN 1856 Lambl of Prague described small filiform processes on the ventricular surface of the semilunar cusps of the aortic valve, and since that time observers have generally referred to them as " Lambl's excrescences." He found them in 2 per cent. of 1000 autopsies and noticed that they were not related to any particular disease : indeed they sometimes appeared in quite healthy hearts. Later writers (Eberth, 1873 ; Koechlin, 1909 ; Krischner, 1927 ; Günzel, 1933) described these structures as occurring commonly on the aortic valve but only rarely on the mitral. The frequent involvement of the mitral valve was not reported until Grant *et al.* (1928) found them in 70 per cent. of 40 normal hearts examined. They regarded these excrescences as having no pathological significance. Ribbert (1924), on the other hand, believed them to be restricted to thickened valves and therefore to be regarded as part of a pathological process.

The presence of these excrescences is still not generally recognised and I was unaware of their existence until, in the course of a study of the mitral valve, my attention was drawn to them and I decided to enquire into their incidence, causation and possible pathological significance. Mitral valves were removed at consecutive departmental autopsies performed on both hospital and coroners' cases, many of the latter being cases of unexplained sudden death. Valves showing bacterial or active rheumatic endocarditis were excluded but those showing chronic thickening were included in order to determine whether the presence of the excrescences bears any relation to this change. Of the 250 valves examined 85 per cent. showed filiform excrescences. The age of the subjects varied from still-births to 87 years. Of the 22 who were less than a year old none showed excrescences, of those between one year and 60 years of age, 90 per cent. were positive, while above the age of 60 all were positive (fig. 1).

If the valve is examined *in situ*, it is only rarely that the fine tags are visible, since they are pearly white or almost transparent and most of them lie flat on the surface of the valve. In order to make them more obvious the valve should be removed from the heart and immersed in water, as was done by Lambl. If it is then examined in a strong light against a black background, the tags can be seen standing out prominently from the surface of the valve (fig. 2). It is helpful to use a binocular loupe magnifying two or three times for this examination.
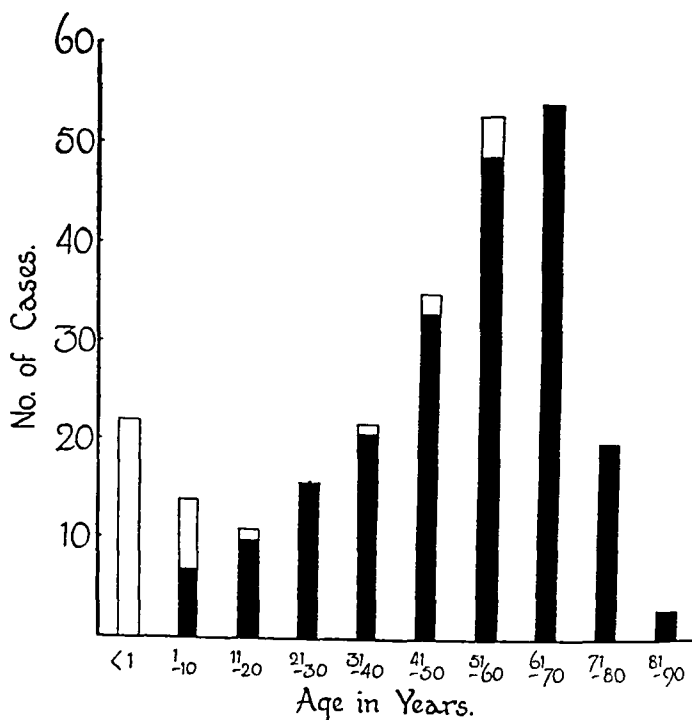
Fig. 1.—The age distribution of the 250 subjects examined. The unshaded areas of the columns represent those in which the mitral valve showed no Lambl's excrescences.

On the mitral valve the excrescences occur only on the auricular surface and from one to a score or more may be found on a single valve. Generally they occur along the line of closure, either forming a row or being grouped in brush-like clusters, chiefly on the summits of the nodular thickenings which are ordinarily present near the free edge of the cusps. They are less frequently found scattered widely over the surface of the valve. They take various shapes; most of them are slender and thread-like (fig. 3) but some are fusiform and a few club-shaped (fig. 4). Their thickness is usually less than 1 mm. and their length varies from 1 to 5 mm., although some are nearer 10 mm. Although they occur quite frequently on valves which

FIG. 2.—A collection of Lambl's excrescences on a mitral valve immersed in water and viewed across its surface.   × 6.

FIG. 3.—A slender excrescence covered by endothelium and having a pale, almost acellular centre with a fine dusting of Sudan III - staining material. Hæmalum and Sudan III. × 35.
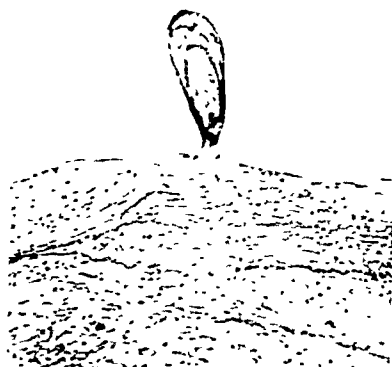
FIG. 4. — A club-shaped excrescence covered by endothelium and containing Sudan III-staining substance which forms a linear pattern in a relatively acellular stroma. Hæmalum and Sudan III.   × 35.

FIG. 5.—A fully organised excrescence containing a tubular coiled structure which, if stained for elastic tissue, would take the stain strongly. In its lower coil the tube can be seen to have a lumen represented by a dark spot. In neighbouring sections the lumen was found to contain Sudan III-staining elements. Hæmalum.   × 155.
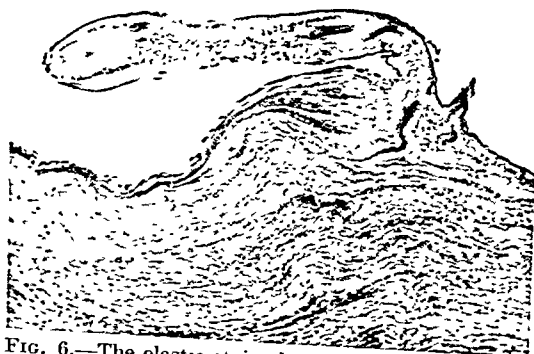
FIG. 6.—The elastic stain shows granules and fibres which form a core extending into the underlying valve. Moore's modification of Weigert's elastic tissue stain. × 45.

Photomicrographs are taken from frozen sections.

LAMBL'S EXCRESCENCES

FIG. 7.—A tag with a dense twisted core of elastic fibres continuous with an elastic lamina of the valve itself. Moore - Weigert elastic tissue stain. ×35.
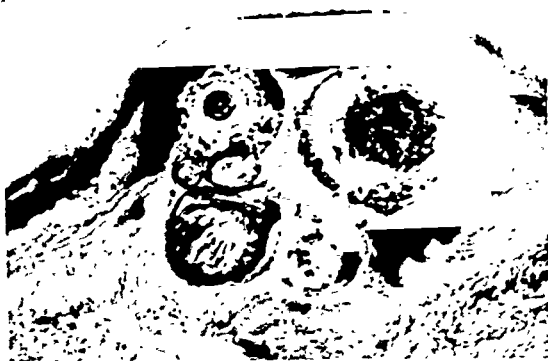
FIG. 8.—Structures beneath the surface of the valve with an elastic tissue architecture similar to that seen in cross sections of some surface tags. Neighbouring sections stained with Sudan III demonstrated the presence of fatty substance in the lumina. Moore-Weigert elastic tissue stain. ×170.

FIG. 10.—Superficial damage to the valve. There is a small encrustation of fibrin on the surface and hæmorrhage into the tissue immediately beneath. Hæmalum. ×140.

FIG. 9.—A very recent fibrin clot adherent to the valve surface alongside a typical excrescence, the core of which can be seen to penetrate a short way into the valve proper. Hæmalum. ×45.

FIG. 11.—Two deposits of fibrin, the darker being the more recent. Both are partially detached from the surface of the valve and resemble Lambl's excrescences in outline and size. Hæmalum. ×30.

LAMBL'S EXCRESCENCES
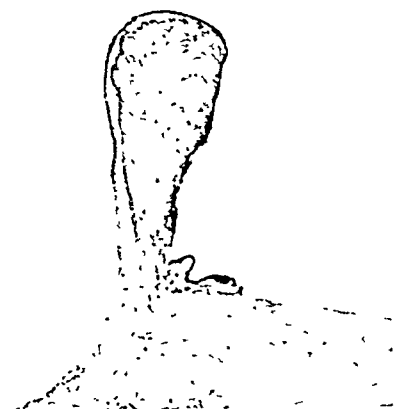
FIG. 12.—Polypoid excrescence consisting mainly of condensed fibrin. Organisation has commenced and is well advanced along the left hand border. Fibroblasts can also be seen growing from the underlying valve into the base of the polyp. Hæmalum. ×17.
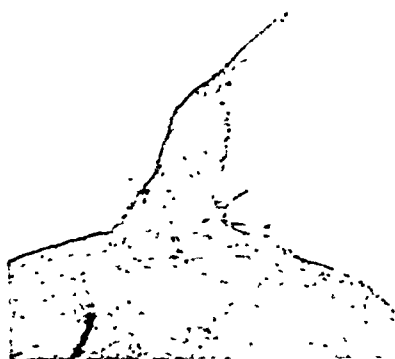
FIG. 13.—An organised excrescence with a thin, almost acellular appendage at its tip representing a more recent addition. Hæmalum. ×25.

FIG. 14.—A thickened valve showing structures which represent pre-existing Lambl's excrescences buried deeply in the valve substance. There is also a neighbouring surface excrescence which has a similar architecture to the buried structures. Moore-Weigert elastic tissue stain. ×35.
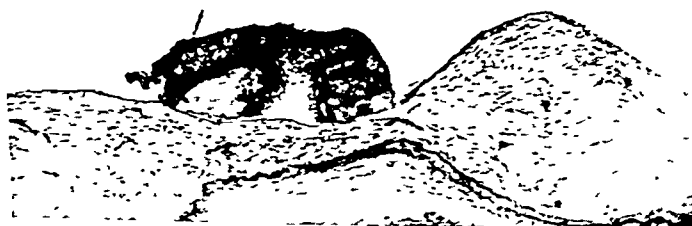
FIG. 15.—Fibrin of different ages is seen, the darker layer on the surface, which is of more recent origin, covers two older, paler masses. Hæmalum. ×40.

LAMBL'S EXCRESCENCES

FIG. 16.—The surface of the valve beneath the hyalinised fibrin deposit is roughened. A few shreds of recent clot form a festoon along the free edge. Hæmalum. ×17.

FIG. 17.—The valve surface beneath the deposit is roughened and there is some subjacent cellular infiltration. Hæmalum. ×17.

FIG. 18.—From a thickened valve. Fibroblasts are growing into the fibrin deposit and endothelium is spreading over its surface from the edge. Hæmalum. ×70.
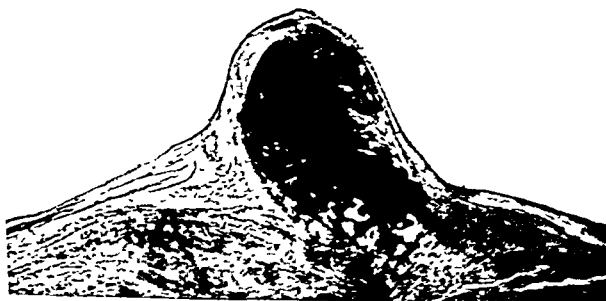
FIG. 19.—Fibrosis of the deposit has advanced most rapidly on the surface, burying some of the fibrin in which organisation is still progressing, represented by the paler areas. Hæmalum. ×27.

otherwise appear normal, they tend to be more numerous on valves which show vascularisation or thickening.

Histologically most of the excrescences consist of cellular connective tissue covered by endothelium (fig. 5). Many also contain a central structure which stains as elastic tissue (figs. 6 and 7) and may be twisted into a bizarre coil (fig. 5). This structure is sometimes seen extending into the substance of the valve, where it may be continuous with one of the elastic laminæ of the valve itself. Cross section often shows these elastic bodies to be tubular, and occasionally smaller concentric cylinders of elastic tissue are found within the main one (fig. 8). A substance staining with Sudan III lies between the layers of elastic fibres. Other excrescences, apparently in an earlier stage of formation, are made up of an almost acellular hyaline material covered by endothelium (fig. 9). These sometimes contain fine Sudanophil granules (figs. 3 and 4).

In Gunzel's opinion these excrescences are formed by a mechanical process and are due to the constant bending and buckling of the valves leading to a tearing of the subendocardial collagen and elastic fibres. A small tag of tissue is thus ripped up from the surface of the valve. Krischner supports a similar but slightly modified view. I have found no evidence in favour of these hypotheses but would support the conclusions of Felsenreich and Wiesner (1916), who considered that the excrescences take origin from surface deposits of fibrin which have become organised. I have found it possible to trace this process in the valves examined.

While looking for Lambl's excrescences I found deposits of fibrin in 18 cases, i.e. in 7 per cent. of the total number. These occur on the auricular surface of the cusps and mainly along the line of closure. Their size ranges from less than half-a-mm. to 3 or 4 mm. across, the smaller being visible only with a hand lens. Some are flat and plaque-like, others hemispherical, pyramidal or pedunculated. These deposits occur either singly or up to about seven on one valve. On some of the valves in which the fibrinous deposits are more obvious the condition has the appearance of so-called " terminal endocarditis." I would suggest, however, that fibrin deposition of this kind is not necessarily terminal but may occur at any time during life. These depositions may well be consequent upon slight intimal damage (fig. 10) caused by the slapping together of the cusps. Their predilection for the line of closure could thus be explained in the same way as rheumatic vegetations, which generally form primarily at this site.

In some instances part of a fibrin deposit becomes lifted from the surface of the valve and then resembles a Lambl's excrescence both in size and in shape (fig. 11). Stages in the organisation of these partially detached fibrin deposits have been seen and the following sequence of events probably leads to the formation of the excrescences.

A layer of intimal cells grows over the surface of the deposit and the enclosed fibrin becomes condensed and hyaline. This hyaline substance, which is originally almost acellular, becomes organised and is replaced by cellular fibrous tissue (fig. 12). As a rule, it also acquires a core of elastic tissue as already described. No blood vessels were ever seen in excrescences, either during the process of organisation or in the fully organised state. Excrescences once formed may increase in size by the addition of further deposits of fibrin, for it is not uncommon to see an organised excrescence bearing at its tip a small acellular hyaline tag covered by endothelium (fig. 13).

Excrescences were never found below the age of one and are therefore not of congenital origin. Their incidence increased with age and they were present without exception over the age of sixty. They do not appear to be indicative of any particular disease and occur on otherwise normal valves as well as on those showing pathological changes. The conclusion is that they represent a normal ageing process and are the result of natural wear and tear.

CHRONIC THICKENING OF THE MITRAL VALVE

During the study of the formation of Lambl's excrescences a similar process of organisation was found in fibrin deposits which had remained fully attached to the valve. This process, occurring repeatedly, gives rise to a progressive thickening similar to that described by Duguid (1948) in the aorta and by Harrison (1948) in the pulmonary arteries. An additional feature of interest is that, in such thickened valves, structures with an architecture similar to Lambl's excrescences can often be recognised partly or completely buried beneath the valve surface (fig. 14). When these buried structures are present, surface excrescences can usually be seen in the neighbourhood and the identical nature of these surface and deep structures seems beyond question.

As stated above, deposits of fibrin were noted on the surface of 7 per cent. of the valves. Although they are sometimes found on normal valves, they are more frequent on thickened valves, suggesting that there may be some relationship between the fibrin deposits and the thickening. Some deposits consist of fibrin of recent origin and have a close reticular pattern with enmeshed red blood cells and leucocytes (figs. 9 and 15); others have lost this pattern and become condensed and hyaline (figs. 15 and 16), and have ceased to take the specific fibrin stains. A hyaline mass of this kind shows a variable tendency to take up fat stains before there is any obvious cellular infiltration from the underlying tissues, a finding similar to that described by Duguid (1946) in thrombi in the coronary arteries. Beneath the most recent deposits there is no histological change in the adjacent tissues of the valve, but later a cellular infiltration appears in the underlying structures (fig. 17) consisting mainly of lymphocytes

LAMBL'S EXCRESCENCES

FIG. 20.—A valve in mitral stenosis. There are several layers of fibrin showing progressive changes, the one on the left being almost replaced by fibrous tissue, but darker staining fragments of fibrin are still recognisable. Hæmalum. ×25.

FIG. 21.—From a case not included in the series to show an organising rheumatic vegetation with a recent fibrin clot superimposed. Hæmalum. ×45.
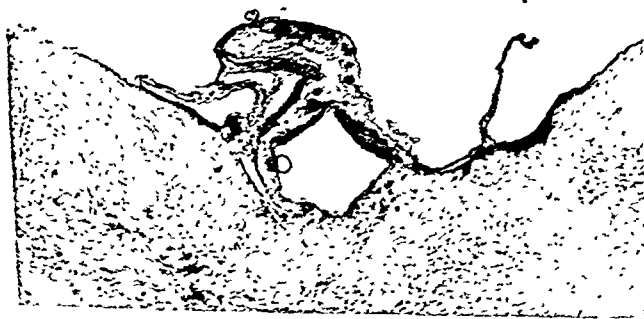

FIG. 22.—The angle of junction of the cusps in mitral stenosis. Organising adhesions partially obliterate the angle between the surface of the cusps. Hæmalum. ×23.

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and macrophages. The line of demarcation between fibrin and valve surface now becomes less distinct and fibroblasts grow from the underlying tissues into the deeper parts of the fibrin (fig. 18). The changes gradually extend until the whole deposit becomes converted into fibrous and elastic tissue, but even when this process is well advanced hyaline fragments derived from the original fibrin frequently persist as evidence of the original nature of the lesion (fig. 19). Endothelium grows over the deposit from the surrounding valve surface (fig. 18) and usually covers it before replacement of the fibrin by fibrous tissue is complete. Blood vessels are never seen in this organising lesion. The end result is a thin layer of newly formed fibrous connective tissue on the surface of the valve. Some valves show several strata in which this process is seen in different stages of development, the changes being invariably less advanced in the more superficial layers of fibrin (fig. 20). Serial deposition of fibrin on the surface of the valve and its replacement by fibrous tissue thus appears to be a mechanism by which the mitral valve progressively thickens.

Two facts already referred to support this contention. One is that the fibrin deposits are more commonly found on thickened valves than on normal : we appear to be seeing stages in a continuous process in which successive depositions of fibrin become organised. The other is the finding of structures having the characteristic architecture of Lambl's excrescences deeply buried in the substance of thickened valves. It would appear that the deposition of fibrin on the valve surface is a recurrent process and that these deposits are successively organised and replaced by fibrous and elastic tissue. Some of the earlier deposits have become partially detached before undergoing organisation, giving rise to Lambl's excrescences. Many of these, while still erect, have become engulfed in successive fibrin deposits with organisation in their neighbourhood.

A sequence of repeated fibrin deposition and consequent organisation may well lead to " senile " thickening of the mitral valve and also contribute to the progressive thickening of the cusps which follows rheumatic carditis and to the genesis of mitral stenosis. After the acute stage of rheumatic fever has subsided organisation of the vegetations gives rise to irregularities and knobs of fibrous tissue. The widespread and successive deposition of fibrin on the surface (fig. 21) to which the valve now becomes prone includes the angles of junction between the cusps. Here the deposits are found bridging the gap and their organisation causes adhesions to form between the opposing surfaces of the cusps (fig. 22). This would appear to play a part in the progressive development of stenosis, as would, in less degree, the thickening of the cusps which results from the organisation of deposits elsewhere on the surface.

Summary

The mitral valve was examined in 250 routine autopsies for the filiform tags known as Lambl's excrescences. They were present in 85 per cent. Their incidence increases with age; they were not found in the 22 subjects below the age of one year but were present in all of the 75 subjects over the age of sixty. They were not associated with any particular disease and it is suggested that they are a manifestation of wear and tear—part of the normal ageing process of the valve.

The structure of these excrescences is described and their method of formation traced. They are shown to be the result of the organisation of partially attached deposits of fibrin on the surface of the valve. Some fibrin deposits were fully attached and lying flat on the auricular surface of the cusps where they were becoming organised. This process appeared to occur repeatedly, leading to gradual thickening of the cusps.

In mitral stenosis, deposits of fibrin undergoing organisation were found on the surface of the valve, including the angles between the cusps. It is suggested that organisation in this situation contributes to the progressive development of the stenosis.

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A CASE OF KALA-AZAR WITH CIRRHOSIS OF THE LIVER AND JAUNDICE

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(PLATES XLV AND XLVI)

THE art of clinical diagnosis is largely based on the recognition of the anatomical alterations and their attendant functional disturbances which result from the pathological process. The bacterial and parasitic infections, owing to their different and often selective effects on their hosts, produce different and often more or less characteristic clinical patterns of disease, which, with the accessory aids of the laboratory, may render a precise diagnosis possible. But the biological inter-relation of host and parasite is not stereotyped. Both are subject to influences of various kinds, and so the clinical and pathological picture varies from case to case. The essential features of the disease, however, are rarely submerged from recognition. Only when extra-ordinary circumstances are present does a particular disease assume a totally different form, so that the clinician is put entirely off his guard. An example of this kind is seen in the case of kala-azar with cirrhosis of liver which we now report.

CASE REPORT

Clinical history

M. S. C. (hospital no. 7699), male, aged 39, married, a native vegetable vender of Szechuan, was admitted to the Central Hospital, Chungking, on 14th January 1943. He complained of epigastric discomfort and yellowish discolouration of sclera and skin of two weeks duration and of marked abdominal distension for one week. Palpitation, dyspnœa and cough with a moderate amount of frothy expectoration, and œdema of the lower extremities, had been present for 4 or 5 days. Two days before admission he had an attack of epistaxis. Fever, abdominal pain, nausea and vomiting were absent. The bowels had moved regularly once a day, the stools being normal in consistency and colour.

Previous general health had been good, but since July 1941 he had had attacks of " malarial fever," each lasting approximately 10 days. The last attack, in June 1942, was controlled by herb medication. There was no history of dysenteric diarrhœa in the past. His social and economic status was poor. He had once enlisted as a soldier and had travelled through Hupeh, Honan and Kiangsi, but returned to Chungking about 1½ years ago. He had been in the habit of taking alcohol in small amount for many years—about two ounces of " white wine " a day.

On physical examination the patient appeared chronically ill and poorly nourished. His mentality was dull and apathetic. Temperature was 37° C. and showed no elevation during the period of hospitalisation. The pulse rate was 100. The skin was dry, rough, muddy and jaundiced. There was no generalised enlargement of lymph nodes, but those in the inguinal region were easily felt. The conjunctivæ were injected and scleræ icteric.

The teeth were dirty and pyorrhea alveolaris was noted at the gum margins. The throat was clear. There were no abnormal findings in the lungs. The heart was normal except for slight upward displacement owing to elevation of the diaphragm caused by the great overdistension of the abdomen. This had resulted chiefly from gaseous distension of the upper abdomen, but an ascites was also present : shifting dullness and a fluctuation wave could be elicited. The abdominal wall was tense and stretched, with dilated superficial veins. The circumference of the abdomen at the level of the umbilicus was 86 cm. Palpation of the liver and spleen was unsatisfactory. There was an indirect right inguinal hernia. Slight pitting œdema of the ankles was present.

Laboratory findings. The blood showed Hb. 7·5 g., R.B.C. 3,600,000, W.B.C. 8400, with neutrophils 74, lymphocytes 23 and monocytes 3 per cent. Icterus index 25 ; Hijmans van den Bergh reaction biphasic (bilirubin 6 units). Stool not obtained. Urine negative except for a positive foam test for bile.

Course in hospital. Owing to the critical condition of the patient only supportive treatment was possible. Duodenal intubation had been tried for relief of the gaseous distension. The patient's condition became gradually worse and he died, comatose, on the second day after admission.

Necropsy

A partial necropsy was done 15 hours after death. Only a small abdominal incision was allowed, and through this the thoracic and abdominal viscera were removed.

Externally the only conspicuous features were the icteric staining of the skin and sclerotics and the protruding abdomen. The peritoneal cavity contained about three litres of clear bile-stained fluid. There were diffuse petechial hæmorrhages in the peritoneum, mesentery and retroperitoneal tissue.

The liver (1210 g.) was rather firm in consistency and yellowish brown in colour. Its surface presented fine granularity, with diffuse whitish flecks of fibrous thickening and a band of adhesion between the right lobe and the diaphragm. On section, the liver was tough and the cut surface showed small irregular lobules of brownish-yellow liver tissue alternating with greyish-white fibrous tissue. A small cavernous hæmangioma was present in the left lobe. The portal lymph nodes were enlarged (2·5×1·5 cm.), but did not appear to have caused interference with the passage of bile through the hepatic and common bile ducts. The gall-bladder showed nothing of note.

The spleen was enlarged (760 g.), extending about 3 cm. below the costal margin in the left mid-clavicular line. It was of doughy consistency and its capsule was tense, the cut surface bulging out, with eversion of the capsule. On section it was congested and deep red in colour, and the Malpighian bodies were indistinct. There was no appreciable fibrosis.

The other internal organs showed no important changes except ecchymosis of the lungs, disappearance of the adrenal cortical lipoid, hæmorrhagic erosions of the gastric mucosa and several small, well-defined superficial ulcers in the mucosa of the colon.

Histology

Liver. Sections from different blocks show extensive strands of young fibrous tissue traversing the liver tissue in all directions, with infiltration by lymphocytes, plasma cells and large mononuclear cells. The normal architecture is much disturbed. In general, the fibrosis appears to begin from the portal tracts and subcapsular tissue, with irregular extensions into the hepatic lobules. Sometimes isolated patches of fibrosis are seen in the midst of liver parenchyma. In more severely affected areas, diffuse fibrosis preponderates over the surviving parenchyma, which appears as irregular islands or strands surrounded and permeated by fibrous tissue and presenting a picture not unlike that of the congenital syphiliic liver (fig. 1). The liver cells show different grades of atrophy. Those towards the periphery of the islands have become shrunken in size and insensibly merge with the fibrous tissue. Elsewhere they show fatty changes of varying intensity. A few liver cells appear hypertrophic. Some of the liver-cell cords contain bile thrombi which, however, are absent from the bile capillaries and larger ducts. The sinusoids in many places are almost obliterated by hyperplasia and hypertrophy of the Kupffer cells and by infiltration with lymphocytes (fig. 2). These various changes would appear to have caused the atrophy of the neighbouring liver trabeculæ. The fibrous areas, apart from being the seat of this cellular infiltration and containing some atrophic liver cells, show many bile canaliculi. On examination with the high power, a remarkable feature becomes immediately obvious. *Leishmania donovani* is everywhere present in the cytoplasm of the phagocytes scattered throughout the fibrous tissue and in the Kupffer cells (figs. 2 and 3). A few atrophic liver cells also appear to be invaded by the parasite and the large mononuclear cells within the branches of portal and hepatic veins and in the hæmangioma are heavily parasitised. In the areas of less extensive cirrhosis a similar state of affairs is seen, but much more liver tissue is preserved. An interesting feature is that while some of the islands of liver cells contain bile thrombi, others do not. In the former, the sinusoids contain fewer parasites and show less hyperplasia of the Kupffer cells.

Spleen. No conspicuous changes are seen in the capsule. The red pulp shows a marked increase of its cells, with a decrease of the red blood corpuscles. The Malpighian bodies were all small in size and hæmorrhages are present around the vessels of the fibrous trabeculæ. With the high power, the cells of the pulp as well as the endothelial cells lining the sinusoids and the large mononuclear cells lying free

in their interior are heavily parasitised with *Leishmania donovani* (fig. 4). The only cells which contain no parasites are the lymphocytes and plasma cells, which are greatly increased in number.

In addition, *Leishmania donovani* is found in the following situations :—(1) in phagocytes and endothelial cells of the portal and pancreatic lymph nodes ; (2) in phagocytes of the alveolar septa of the lungs ; (3) in the endothelial cells lining the glomerular capillaries of the kidneys ; and (4) in the mononuclear cells of the mucous membrane of the small intestine, though none was found at the edge of the small ulcers.

DISCUSSION

To summarise, the case is one of heavy infection with *Leishmania donovani* of the liver and spleen, and, we may assume, of the bone-marrow also. But the particular point of interest in this case is the cirrhosis of liver. In the literature we can find but few references to its occurrence in kala-azar. Hu (1936) noted a moderate increase of fibrous tissue in the portal tracts of chronic cases of Leishmaniasis and regarded the condition as one of mild biliary cirrhosis. Gabbi and Abate (quoted by Brahmachari, 1928) also mention biliary cirrhosis in kala-azar. A remarkable form of diffuse cirrhosis was described by Rogers in 1908-09. He depicted the liver as tough in consistency and with a smooth surface. Microscopically it was a diffuse " intercellular cirrhosis ", in the connective tissue of which shrunken parasites were visible under the high power. Rogers's description strikingly resembles the anatomical features of the present case. Apparently this type of cirrhosis of the liver in kala-azar is a rare occurrence, as we can find no other reports in the literature. The case of ictero-ascitic leishmanial hepatitis described by Bonan and Mamou (1937) might conceivably be of this kind, but in the absence of laboratory proof it cannot be accepted unreservedly.

We would point out here that the present case is not one of pre-existing atrophic cirrhosis with superimposed leishmanial infection, as one might think at first sight. The anatomical changes of the usual form of atrophic or diffuse nodular cirrhosis are characteristic : there is no biliary obstruction leading to jaundice : though its ætiology is obscure, the pathological process is slow and chronic, probably resulting from various assaults with intervals of quiescence which allow repair and regeneration to occur. On the contrary, the morbid anatomy of the present case indicates a generalised active process of recent development and rapid evolution. The fibrosis is extensive and diffuse and the fibrous tissue generally is uniformly young. This process is also faithfully represented in the unusual clinical manifestations. Clearly this was an extraordinary type of cirrhosis.

Apart from the close anatomical resemblance to the case described by Rogers, we have reason to believe that the leishmanial infection in the present case was responsible for the pathological changes in

LEISHMANIASIS AND CIRRHOSIS OF LIVER
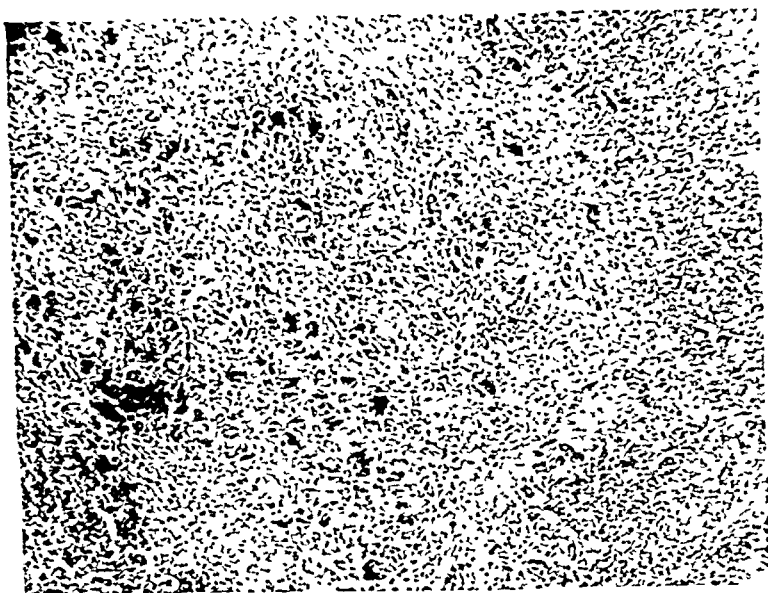
FIG. 1.—Liver: low-power magnification showing the general appearance of the cirrhosis. The surviving liver cells appear in small isolated groups embedded in fibrous tissue which is infiltrated with small lymphoid cells.
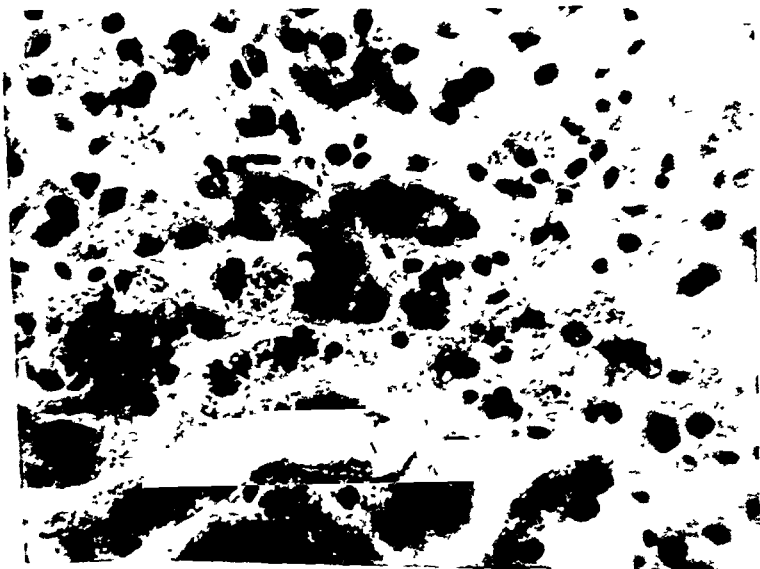

FIG. 2.—Liver tissue from one of the less cirrhotic areas. The liver cells still appear in trabeculæ. The Kupffer cells lining the sinusoids, particularly in the centre of the field, are hypertrophic and contain numerous Leishman-Donovan bodies in their cytoplasm. They practically block up the sinusoids.

FIG. 3.—Liver : high-power magnification showing numerous parasitised cells in the cirrhotic area. Two bile canaliculi are seen in the middle field.
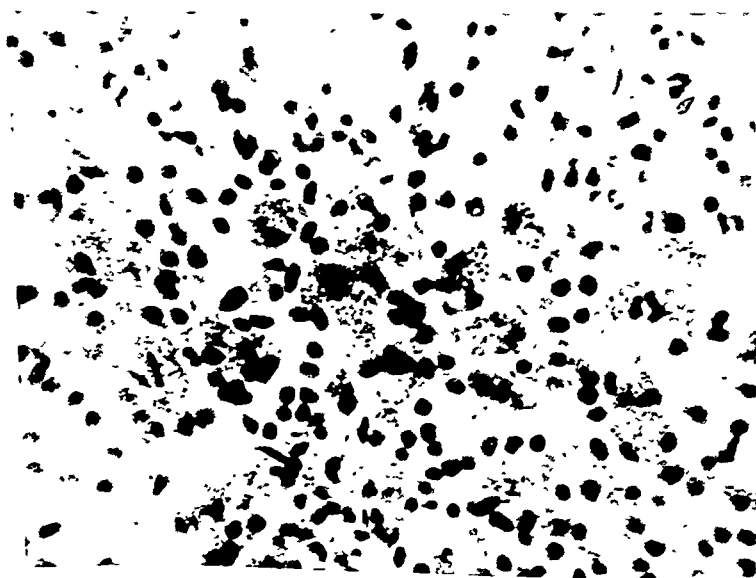
FIG. 4.—Spleen : high-power magnification showing numerous large phagocytes loaded with Leishman-Donovan bodies. The lymphoid cells are free from parasites.

the liver. The parallelism between the severity of the lesion and the abundance of the parasites in the same areas of liver strongly suggests the relation of cause and effect. If this is so, how did the parasitic infection bring about the cirrhotic change ? At first sight the finding of the parasite in a certain number of damaged liver cells would suggest that the parenchymal destruction was the direct result of the invasion and the fibrosis a subsequent development. The following considerations, however, do not support this view. (1) Parasitisation of the liver cells is only an occasional and exceptional finding. Should direct invasion have been responsible for the destruction of the larger areas of liver cells we should expect a more generalised parasitisation of the atrophic and disintegrated liver cells in and around the cirrhotic areas. (2) *Leishmania donovani* is almost exclusively a parasite of the reticulo-endothelial cells. So long as these cells were not exhausted, the parasites, after having multiplied in and caused rupture of the Kupffer cells, would soon be taken up again by new phagocytes. Only when hyperplasia of these cells reached such a state as to block the sinusoids and involve large areas would the parasite be likely to invade the hepatic parenchyma. (3) *Leishmania donovani* has been found (Hu) in heavily infected cases of kala-azar without cirrhosis. It would seem, then, that in the present instance some other pathogenic mechanism was operative.

The history shows that the patient was a chronic alcoholic, and alcohol is generally believed to be one of the principal causes, direct or indirect, of atrophic cirrhosis of the liver. But the known effect of alcohol on the human liver is the production of an enlarged and fatty organ. Experimentally, alcohol alone does not produce cirrhosis in animals. Only when it is combined with some bacterial or chemical poison can it be shown to produce this effect. It is therefore most unlikely that a chronic tolerant alcoholic like our patient, who remained well for years, could, without apparent cause, develop a rapidly fatal cirrhosis. On the other hand, chronic alcoholism might well be a predisposing factor rendering the liver cells more vulnerable to the parasitic toxins. That *Leishmania donovani* produces some sort of toxin is evidenced by the clinical symptoms and pathological changes encountered in all cases of kala-azar.

The parasitic infection in the present case had probably been in existence for some two years, for Chungking is not an endemic area of kala-azar and the patient must have been infected during the time he was a soldier, while the symptoms of hepatic catastrophe and the anatomical changes in the liver both indicated a recent development. It would seem that either the incubation period was unusually long or that the infection was a mild one and had long remained latent. The sudden flare-up of parasitic activity late in the infection would suggest some additional factor which lowered the tissue resistance.

In view of these considerations, we may reasonably infer that only

a few parasites were originally present in the liver and spleen, and probably also in the bone marrow, and that the relation of host and parasite was more or less in a state of equilibrium. As the balance tilted over in favour of the parasites, the latter became capable of more and more unopposed multiplication in the body. In the liver, not only were the existing parasites increased in number, but more of them were transported by the portal and arterial blood. The sinusoids in the periphery of hepatic lobules were thus destined to be the first filter and more and more parasites were accumulated there. The Kupffer cells, stimulated by the parasites, underwent progressive hyperplasia with an intensity directly proportional to the number of parasites. When a sufficient concentration of toxin had been reached, not only the parasitised Kupffer's cells but the liver cells also would suffer damage. Progressive fibrosis would naturally follow parenchymal destruction, with interruption of both biliary and portal flow : hence the jaundice and ascites.

It will be recalled that hyperplasia of the Kupffer cells and infiltration by lymphoid cells were present everywhere but were most pronounced around the cirrhotic areas. Here the sinusoids were practically blocked up. The same state of affairs must have existed in the cirrhotic areas before destruction of the hepatic parenchyma and fibrosis occurred. Blockage of vessels would deprive adjacent liver cells of their blood supply and the consequent fatty and atrophic changes would lead to final disintegration, apart altogether from the toxic effect of the *Leishmania donovani*. We believe, however, that this also was a pathogenic factor.

While these hypothetical considerations would explain very well the anatomical picture of the liver, we must not lose sight of another possibility. The history of jaundice and ascites appearing in the short period of 3 weeks and the histological changes in the liver might well be attributed to acute infective hepatitis. In fact we are unable to exclude this possibility as a terminal event, though equally unable to prove its existence. This question must be left unanswered but kept in mind. If acute infective hepatitis were responsible for the extensive and rapid destruction of the hepatic parenchyma, it might also have accelerated the multiplication of the parasites and this in turn might have lowered the tissue resistance. That this form of cirrhosis has not been more often described, in view of the fact that kala-azar is such a prevalent infection in many countries, gives food for thought, and we may well ask the question, is this form of cirrhosis the combined effect of leishmanial and viral infection ?

Clinically the signs and symptoms in this case were entirely those of portal and biliary obstruction. The course of the disease was so short that we were led to think of a possible malignant process. Indeed, nothing in the clinical findings had suggested kala-azar. Such a complete departure from the usual picture, now adequately explained by the extraordinary pathological process in the liver, is

not only interesting in itself, but highly instructive in the differential diagnosis of cirrhosis of the liver with portal obstruction and jaundice.

SUMMARY

1. A case of heavy visceral leishmaniasis associated with diffuse atrophic cirrhosis of the liver and jaundice is described.

2. The liver was characterised by reduction in size, a rather smooth surface and tough consistency. Histologically, there is diffuse cirrhosis with slight variation in intensity in different parts of the organ. *Leishmania donovani* was present everywhere, in the cirrhotic areas as well as in the hyperplastic Kupffer cells of the surviving liver tissue.

3. The pathogenesis of the cirrhosis is traced to this parasitic origin and the mechanism of its production is discussed.

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* The longest incubation period known was 33 months.

meso-appendix, which joins it to the ileum, in the extensive vascular anastamosis between meso-appendix and ileum, and in its thinner outer fibromuscular layer and thicker middle lymphoid layer.

Experimental procedures

All operations were performed under open-ether anæsthesia and with strict aseptic technique.

1. *Production of mucocele of the appendix.* Following the method described by Grodinsky and Rubnitz (1941) the appendix was exposed and its lumen irrigated with 60-80 c.c. of 0·9 per cent. saline by means of a syringe and needle. The appendix was then ligated about 2 cm. from its base without including the blood vessels. A 2 mm.-thick string was used for ligature, as ordinary thin silk sutures cut through the wall, with re-establishment of the lumen. In later experiments 2 c.c. of a mixture of sulphaguanidine and penicillin were injected into the lumen before ligation. This reduced the incidence of infection and so increased the chance of mucocele production. The abdomen was closed in two layers. Laparotomy was again performed if no cystic mass could be palpated in the abdomen two weeks after the first operation and a ligature was re-applied if no mucocele was apparent.

Some of the mucoceles were left undisturbed for varying lengths of time up to three months, the animals being killed at intervals. With others the abdomen was re-opened and the wall of the mucocele artificially ruptured to allow escape of its contents into the peritoneal cavity. Such animals were eventually killed at intervals up to six weeks. Five c.c. of the sulphaguanidine-penicillin mixture were introduced into the peritoneal cavity of four rabbits. Several small nodules showing foreign-body giant-cell reaction developed after two weeks, leading eventually to tiny scars.

2. *Autoplastic transplantation of the mucosa of the appendix.* The distal 3 cm. of the appendix were resected after ligation and the ligated end of the proximal portion was cauterised with phenol. The mucosa of the resected portion was stripped off and uniform fragments, 2 mm. in diameter, were cut by means of a cork-borer. After washing in a sulphaguanidine-penicillin mixture they were grafted on to various sites in the peritoneal cavity of the same rabbit. About fifteen implants were made at one time. The animals were killed at different intervals up to six weeks.

3. *Intraperitoneal injection of mucocele contents sterilised with chloroform.* The contents of some mucoceles were evacuated into a sterile flask, saturated with chloroform and allowed to stand for twenty-four hours. It was hoped in this way to kill both bacteria and cellular elements in the sample, while preserving its physico-chemical character. The material was finally transferred to a sterile Dreschel bottle and the chloroform evacuated. Culture of this material disclosed no bacterial contamination. About 10 c.c. of the sterile mucoid contents were introduced with aseptic technique through a small abdominal incision into the peritoneal cavity of a healthy rabbit. Attempts to remove the mucocele intact so that its contents after sterilisation could be inoculated later into the same animal failed because of adhesions around the dilated appendix. Filtration through a Seitz filter was also unsuccessful. The animals were allowed to survive up to six weeks.

Histological technique

The tissues were fixed in 10 per cent. formol-saline, Helly's fluid and absolute alcohol. They were dehydrated in graded alcohols, cleared in chloroform and embedded in paraffin. Sections were stained with Ehrlich's acid hæmatoxylin and eosin, and Weigert's iron hæmatoxylin and van Gieson's stain. The alcohol-fixed sections were stained with mucicarmine.

RESULTS

Mucocele production

Of fifteen rabbits employed, one developed gangrenous appendicitis and another acute appendicitis with purulent peritonitis ; one died 24 hours after operation without obvious cause ; several required re-ligation of the appendix.   In successful cases the appendix was transformed into a sausage-like, globular or pyriform structure ranging from 5 to 15 cm. in length and 2 to 3 cm. in transverse diameter (*cf.* figs. 1 and 2). The external surface was congested and usually densely adherent to surrounding structures, possibly owing to some leakage of contents. Diverticulum formation was frequently observed, indicating the weakened state of the wall. Excluding cases where the wall was artificially ruptured, none showed a definite perforation, although there were several in which this may have happened with subsequent closure by adhesions. Such was frequently the case in animals with artificial perforation. The wall varied in thickness, being sometimes very thin and atrophic, at other times somewhat thicker than normal. No definite relation could be established between the size of the mucocele and the thickness of its wall, and the duration of the obstruction.

The contents of the mucocele varied greatly in character. In some animals it was colourless and translucent and either thin or viscid ; in others it was thick and gelatinous.   Only these cases justify the name of mucocele. Occasionally globules of more tenacious mucoid material were found in the sac, but unfortunately the contents were not cultured. In a proportion of the animals greyish-white opaque pultaceous material occupied the lumen. Direct smears then showed numerous Gram-positive and Gram-negative bacilli, with large numbers of pus cells and disintegrating necrotic cells. Obviously infection had been superimposed on obstruction and the condition should more appropriately be called pyocele or empyema of the appendix.   Portions of shed necrotic mucosa were present in some cases, and in two, some dark blood clots were found.

Artificial perforation of the mucocele produced peritoneal deposits within 2-4 weeks, and two of the mucoceles which were left intact showed similar lesions. The deposits, which varied in size from 0·25 to 2·5 cm. in diameter, were commonly distributed in the neighbourhood of the cystic appendix. Small deposits were usually globular in shape ; large ones occurred as irregular masses. They were firmly adherent to the underlying structures and some were encapsulated by fibrous tissue. The nature of the contents differed greatly ; in general it was semi-solid and greyish, with central necrosis, but deposits associated with pyocele of the appendix resembled chronic abscesses with dense capsules. Only in a few were the deposits translucent and mucoid in character.

Autoplastic peritoneal transplantation of mucosa from the appendix

Grafting of mucosa from the appendix into the peritoneal cavity was successful in all seven animals, though more " takes " were found in some animals than in others. They appeared as small indurated nodules 0·25 to 0·5 cm. in diameter and were firmly adherent to the underlying tissue. Only one graft showed a tiny cyst with watery contents. None of the grafts gave gross evidence of mucoid secretion.

Intraperitoneal injection of mucocele contents

After the intraperitoneal administration of chloroform-treated mucocele contents, one rabbit died in 24 hours without apparent cause. Examination showed plaques or irregular masses of gelatinous material 2-3 cm. in diameter in various sites within the peritoneal cavity, attached by fibrin to the underlying structures. Other animals killed 7-42 days after injection presented similar opaque and greyish deposits but these were firmly adherent to the underlying tissues. In addition, some encapsulated mucoid material was found. A few deposits had long fibrous pedicles.

Histological observations

Wall of mucocele of appendix. In three appendices the mucocele wall has become a thin, fibro-muscular structure (fig. 7) with scattered remnants of mucosa. In the remainder the outer sero-muscular layer, which normally makes up about 2 per cent. of the thickness of the wall (fig. 3), is hypertrophic and constitutes nearly 90 per cent. of the entire wall (figs. 4-6). The middle lymphoid layer is greatly reduced in amount or has entirely disappeared. The inner glandular layer (usually 25 per cent. of the wall) has either disappeared completely or is compressed so that the glandular tissue is reduced to a single investing layer of epithelial cells (fig. 6). These cells, however, are still secreting mucin. In other cases, especially in artificially ruptured mucoceles, the glandular layer has lost its normal complex structure and goblet cells are prominent (fig. 4). Inflammatory reaction in the wall of the appendix is usually not marked. The mucocele contents fail to stain with mucicarmine.

Peritoneal deposits. Two types of peritoneal deposits develop, although intermediate forms are found. The more common form presents a dense fibrous capsule or trabeculæ enclosing granular necrotic material (fig. 8). Sometimes mucin threads are also present. The capsule and trabeculæ are infiltrated, focally or diffusely, with histiocytes, lymphocytes and foreign-body giant cells (fig. 8). Here and there are some epithelial-like cells arranged in rows or even in a glandular pattern. Their origin cannot be established with certainty. The second type displays a picture of organisation. Eosinophilic

EXPERIMENTAL MUCOCELE OF APPENDIX

FIG. 1.—Normal rabbit appendix. $\times \frac{5}{8}$.

FIG. 2.—Two mucoceles of the appendix. $\times \frac{3}{7}$.
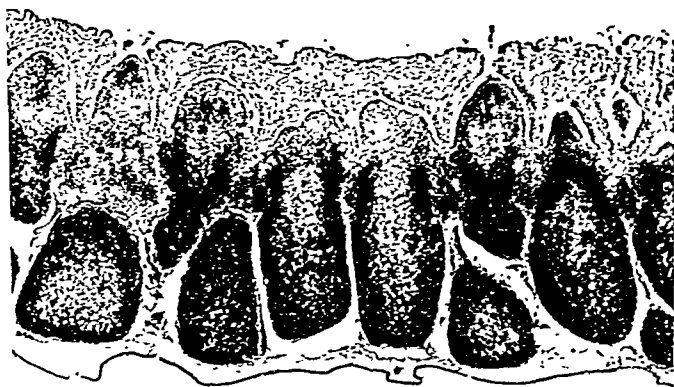
FIG. 3.—Wall of normal rabbit appendix, showing thin sero-muscular layer and thick lymphoid layer. Hæmatoxylin and eosin. $\times 18$.

FIG. 4.—Wall of mucocele, showing greatly hypertrophied sero-muscular layer and atrophic lymphoid layer. The glandular layer is less complex in structure and goblet cells are prominent. Hæmatoxylin and mucicarmine. $\times 18$.
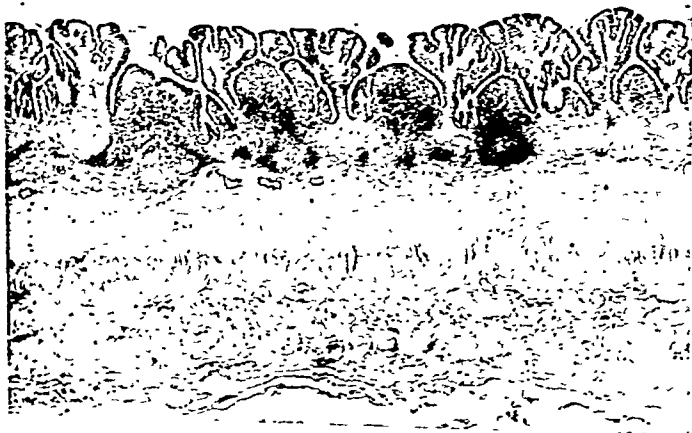
EXPERIMENTAL MUCOCELE OF APPENDIX
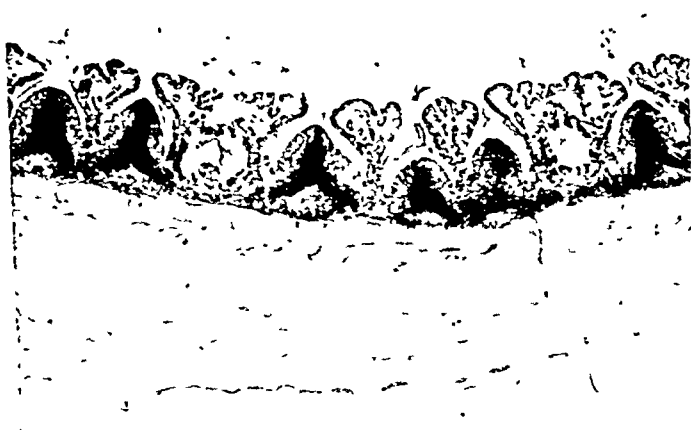
FIG. 5.—Mucocele wall, showing more advanced changes than in fig. 4. Hæmatoxylin and eosin. × 18.

FIG. 6.—Mucocele wall, showing disappearance of lymphoid tissue and the glandular layer reduced to a single investing layer. Hæmatoxylin and eosin. × 18.
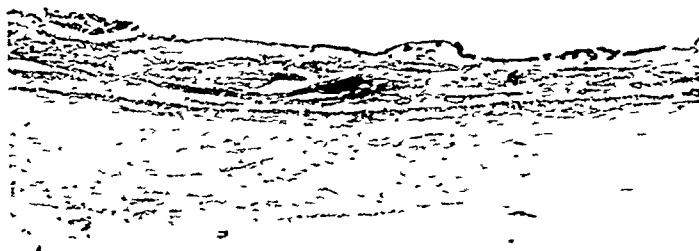
FIG. 7.—Mucocele wall almost entirely transformed into fibromuscular tissue. Hæmatoxylin and eosin. × 18.

FIG. 8 — Peritoneal deposit : section showing granular necrotic material surrounded by dense fibrosis with active inflammation. Haematoxylin and eosin. × 45.

FIG 9 —Peritoneal deposit section showing mucoid material in process of organisation One or two foreign body giant-cells are present in the right upper corner Hæmatoxylin and eosin. × 20.
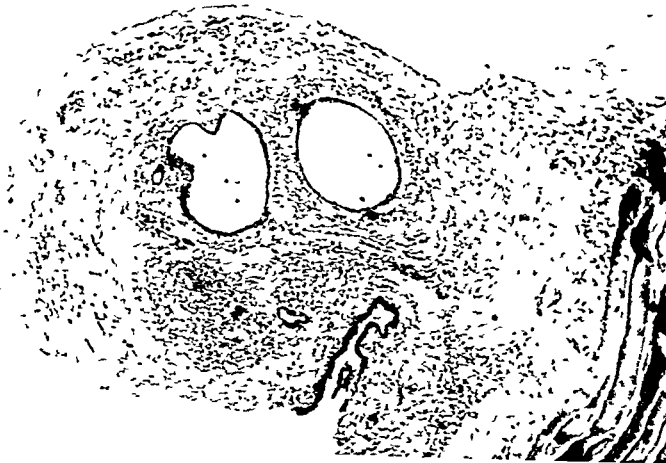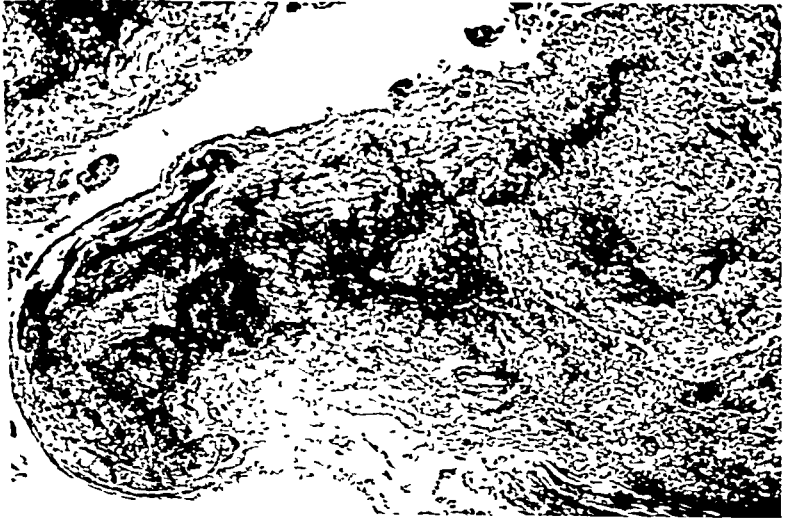
FIG 10.—Peritoneal graft of mucosa of appendix. Hæmatoxylin and eosin. × 45.

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FIG. 11.—Sterilised mucocele contents injected into peritoneal cavity: section showing organisation of gelatinous material. Hæmatoxylin and eosin. ×65.
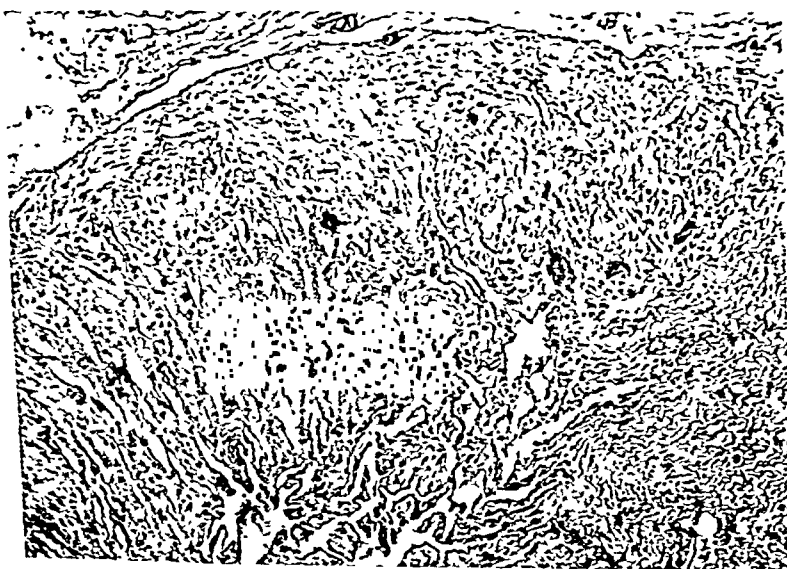
FIG. 12.—Sterilised mucocele contents injected into peritoneal cavity: section showing completely organised mass. Hæmatoxylin and eosin. ×60.

material is surrounded by young fibrous tissue containing numerous fibroblasts and often newly formed capillaries are observed invading the mass. Both the eosinophilic substance and the fibrous tissue are infiltrated with histiocytes, lymphocytes and sometimes foreign-body giant cells (fig. 9). Epithelial-like cells are also noted. Some deposits are completely organised. Mucicarmine fails to stain the homogeneous matter.

Fate of peritoneal transplants of mucosa of appendix. The implanted epithelial glandular tissues are surrounded by dense fibrous tissue, sometimes with giant cells, and often with focal collections of histiocytes. Lymphoid follicles are present in some grafts. There are 2-4 glandular structures in each section and some glands show an irregular outline with dilatation of the lumen. The latter is empty or contains a little pink granular debris or dead cells. The lining cells are columnar, cuboidal or flattened. Some transplants give evidence of glandular budding but not of cell mitosis. Only one transplant of one week's duration contained a few goblet cells with mucin. In none of the others is there any sign of mucous secretion in the glandular cells (fig. 10). True mucous cyst formation is never found. Sections of the cystic graft mentioned before show flattened lining cells free from mucous secretion.

Lesions resulting from the intraperitoneal injection of chloroform-treated mucocele contents. Two main lesions developed. The first resembles the second type of lesion associated with peritoneal deposits from the mucocele. An eosinophilic homogeneous material is surrounded and invaded by young fibrous tissue infiltrated with histiocytes, lymphocytes and often huge giant cells. The inflammatory reaction is seldom intense. This homogeneous material fails to stain with mucicarmine. In some parts, mucinous threads and necrotic foci are also observed (fig. 11). The second and commoner lesion discloses complete replacement of the injected material with young fibrous tissue containing numerous fibroblasts and little collagen (fig. 12).

DISCUSSION

Experimental ligation of the appendix most often results in acute appendicitis and peritonitis, although sometimes there follows distension of the lumen with muco-purulent material (Adrian, 1901; von Lichtenberg, 1906; Phemister, 1915; Behan, 1921). However, Wells (1936-37) was able to produce mucocele in rabbits by ligating the base of the appendix without disturbing its vascular supply. He claimed that appendicitis followed only when the mucosa was injured. Wangensteen and Bowers (1937) and Wangensteen *et al.* (1937) reported that obstruction of the cæcal appendage of dogs is usually well tolerated, provided the organ is irrigated before closure. More interesting results were obtained by Naeslund (1928-29) in newborn rabbits in which bacterial invasion of the

intestinal tract had not yet occurred. He divided the appendix after ligating it near its base, leaving the distal segment open. Granulation tissue closed the gap and a mucous cyst of the appendix developed. In some animals small cystic mucous deposits formed on adjacent structures, invested either by epithelial cells or fibrous tissue infiltrated with macrophages, lymphocytes and giant cells. Nests of epithelial-like cells were sometimes present. Mucosal epithelium from the cystic appendix grew into the granulation tissue and the appendix wall or occasionally extended to the serosal surface through the opening. Naeslund also transplanted the mucous coat of the appendix, directly or after tissue culture, with bone marrow extract and the contents of pseudomucinous ovarian cysts into other rabbits. The cells failed to survive except in one doubtful example. Naeslund did not commit himself as to the origin of peritoneal cystic masses. Rubnitz and his associates (Grodinsky and Rubnitz, 1941 ; Rubnitz and Hermann, 1943) ligated the appendix after irrigating its lumen and produced peritoneal deposits. They claimed that pseudomyxoma is an inflammatory lesion. They also injected into rabbits by various routes mucocele contents devitalised by heating and standing and they administered mucocele contents to different species of animals, assuming that heterologous transplants would not survive. Secondary deposits developed and the authors concluded that it was the mucoid material and not the cellular element which was responsible for pseudomyxoma peritonei. Donat (1885), Auché and Chavannaz (1898) and Tóth (1905) injected the contents of ovarian pseudomucinous cysts into the peritoneal cavity of rabbits and dogs and found that mucoid material was either entirely absorbed or encapsulated by granulation tissue.

My experiments do not substantiate the commonly accepted contention that the gelatinous masses of pseudomyxoma peritonei are products of appendix epithelial cells spilled into the peritoneal cavity. A survey of the literature reveals general agreement among authors on the scanty nature or entire absence of epithelial-like cells in the gelatinous masses. That such cells originate from the appendix is also open to doubt in view of the fact that the serosal cells of the peritoneum can assume a cuboidal or columnar form and may even result in tubular formations after stimulation (von Brunn, 1901 ; Cunningham, 1926). In my animals, transplants of mucosa from the appendix survived but none showed evidence of secretory activity. Of course it may be argued that the implanted cells are rapidly transformed into masses of mucoid secretion and the difficulty in finding them is the same as in mucoid carcinoma, where the cellular elements virtually dissolve up in the mucus (McCrae and Coplin, 1916 ; Willis, 1934). It is true that some cases of human pseudomyxoma peritonei originate from the rare mucoid carcinoma of the appendix but it is equally true that many cystic appendices reveal no evidence of malignant growth.

On the other hand, the results of my experimental investigation present strong support for the opinion of Trotter (1910) and others who consider that psuedomyxoma peritonei is a type of foreign body peritonitis. Thus mechanical and chemical irritation of the peritoneum by the escaped mucocele contents causes a reactive inflammation which leads to slow removal of the gelatinous material by absorption, organisation and encapsulation. The reaction falls into line with the peritoneal response to various foreign bodies (Marchand, 1889; Stewart, 1914-15; Florey and Carleton, 1926; Haythorn, 1933; Thomas, 1936).

If one accepts this view, then constant production of mucus with its continuous passage into the peritoneal cavity becomes an essential part of the mechanism. The glands of the appendix must be secreting actively a richly mucous product. If the intraluminal pressure becomes too great the glands atrophy and disappear. It would seem that this is often prevented by intraperitoneal extravasation of the mucocele contents before the pressure becomes excessive and harmful to the epithelial lining. The quantity and nature of the extruded mucoid material, the extent of the adhesions around the cystic appendix and the state of the mucocele wall which determines the escape by rupture or perforation, or even through microscopic openings, are also important. Furthermore, the relative sterility of the appendix lumen, the intensity of the peritoneal reaction and the possibility of absorption by lymphatic vessels are additional factors that may decide the ultimate outcome. It is therefore not surprising that the condition is a rare one, although it is possible that minor degrees escape attention because of the operation of these factors.

Details of twelve cases collected by Naeslund correspond well with those of my experimental investigation. However, a survey of pertinent literature and examination of the specimens in the pathological museum of University College Hospital Medical School indicate some divergencies. The peritoneal reaction to the gelatinous extravasate in human cases is often extremely delicate, appearing merely as grape-like cystic projections from the peritoneum with little microscopic evidence of irritation. Only fine fibrous strands are seen subdividing the gelatinous masses and inflammatory cells are insignificant. In some instances the mucoid material may be lying free without encapsulation (Fraenkel, 1901). Some, too, present an appearance suggestive of encysted mucoid material in a blocked lymphatic vessel or in the fibrous stroma of the peritoneum with little surrounding inflammation. In contrast the experimental examples demonstrate vigorous organisation and the peritoneal deposits consist chiefly of an exudate with a relatively small mucoid component. No satisfactory explanation for this difference could be found. Whether it is partially due to a difference in the physico-chemical characters of the mucin is difficult to say without further study. Olitzki (1948) mention that different preparations of mucin vary in their action

on bacteria and presumably this might influence the reaction of other types of cells.

Cases are also reported in which removal of the original mucocele does not arrest the pseudomyxomatous process, which continues with unabated energy to produce more gelatinous material in the peritoneal cavity (*e.g.* Michaëlsson's case 3, 1920-21). Such observations are undoubtedly in favour of a malignant origin and quite likely prompted most authors to accept a cellular origin. Woodruff and McDonald (1940) after examining 146 cases of cystic appendix concluded that ten were grade 1 cystocarcinoma and suggested that these resulted from the malignant conversion of a benign mucocele. Their histological description, however, recalls papillomatous change rather than malignancy. Whether such a tumour is capable of proliferation and secretion after transplantation without becoming malignant is still conjectural.

Another interesting problem for future study is the frequent coexistence of appendix cyst, ovarian pseudomucinous cyst and pseudomyxoma peritonei in females. Ries (1924) has shown that this is unlikely to be the result of metastasis. He suggests that some of the recurrent types of pseudomyxoma peritonei in females may be due to an overlapping lesion that has been overlooked during operation.

SUMMARY

1. If uncomplicated by infection, experimental obstruction of the appendix leads to mucocele with extravasation of its contents and foreign body peritonitis.

2. Autoplastic peritoneal transplants of appendix mucosa " take," but the epithelial cells show no evidence of secretory activity.

3. Intraperitoneal injection of chloroform-treated mucocele contents also gives a foreign-body peritonitis.

4. The relation of the above findings to human pseudomyxoma peritonei is discussed and it is suggested that most instances of this condition are inflammatory in nature, although there are some cases with malignant features which suggest a cellular origin. In such, the implanted tumour cells continue to produce mucin which is discharged into the peritoneum.

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A CASE OF INTRADURAL SPINAL LIPOMA

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(PLATES LI AND LII)

INTRASPINAL lipomas, excluding those representing merely masses of displaced fat associated with spina bifida, constitute only about one per cent. of all primary intraspinal tumours. They fall into two distinct groups, intradural and extradural.

The *intradural lipomas* are of greater interest because of their occurrence in a region generally considered to be devoid of fat cells and because of their occasional association with anomalies of development. Ehni and Love (1945), in a comprehensive review of the literature, collected only 29 cases of intradural lipomas, including 4 of their own from the Mayo Clinic. They discussed the various theories suggested to explain the occurrence of lipomas within the dura mater, their intimate relation to and probable origin within the pia, and their predilection for sagittal locations, for the cervico-thoracic junction of the spinal cord and for the posterior surface of the cord. The symptoms usually appear at one of three age periods—before the third year, in adolescence and about the age of forty—and they are often present for many years before producing serious disability. It is probable that the tumours originate in fœtal life.

The *extradural lipomas*, on the other hand, are not congenital; they occur at all ages and usually have a short history. There is no characteristic segmental distribution and they are often associated with multiple lipomas or obesity.

The present case is reported because of the rarity of intradural lipomas. The occurrence of a severe kypho-scoliosis at the same segmental level as the tumour obscured the pre-operative pathological diagnosis.

CASE REPORT

M. C., a male aged 40 years, was admitted to the Leeds General Infirmary as a case of recent spastic paraplegia associated with kypho-scoliosis and bronchiectasis of many years duration.

Clinical history

Previous history. At 3 years of age he had pneumonia and he was educated at an open-air school because of a "weak chest." At 16 a "lump" developed on the back, and he stated that the spinal deformity had remained unchanged since then. At 20 he had an illness consisting of gross weakness and sensory loss of the legs, very similar to the present attack except that it cleared up spontaneously after a few months. He stated that the attack began with cramps in the legs and a sensation of coldness which gradually ascended from the feet to the upper chest; this was followed by sensory loss and weakness of the legs, right more than left, and after three months he was unable to walk. After some months in bed recovery started spontaneously and within a year he was again walking normally and had returned to work. He remained well and able to work as a wool bundler until the onset of the present attack.

Recent history. In August 1947, five months before operation, he again noticed cramps in the calves and numbness and weakness in the legs, first the right, then the left. These symptoms started while he was in another hospital under treatment for bronchiectasis, his admission there having been necessitated by a single hæmoptysis one month previously. After his return home, the leg symptoms progressed and in September he had difficulty in walking; the left foot was weak and the right was paralysed. There was also increasing difficulty with micturition. There was no spinal or root pain. Eventually the paraplegia became complete.

Examination on admission showed that the general condition was good, but he was coughing up a certain amount of foul-smelling sputum. There was gross upper dorsal kypho-scoliosis with the convexity to the right; it was not tender. The cranial nerves and arms were normal. The legs showed complete paraplegia with moderate spasticity, sustained bilateral knee and ankle clonus and extensor responses. There was complete loss of all forms of sensation up to a sharp level at T4, absent abdominal reflexes and retention of urine with overflow incontinence.

Lumbar puncture showed a complete block on jugular compression. The cerebrospinal fluid was clear and straw-coloured; it contained 1300 mg. of protein per 100 c.c. and 1 cell per c.mm.

X-ray examination showed that the summit of the kyphosis was at T4-6; there was no evidence of active bone disease or of paravertebral abscess and no gross changes were seen in the lungs.

Clinically, there was thus a complete transverse spinal-cord lesion and its level corresponded to the summit of the kyphosis; also, there was no doubt about the presence of a spinal block. In order to avoid invoking a double pathology it had to be assumed that the cord compression was due to the kypho-scoliosis, although the latter had caused no trouble for the past twenty years until recently, and there had been no pain. It was, however, difficult to believe that the spinal curvature of itself could produce a complete block, because the gibbus was fairly rounded and not sharply angulated. There was no evidence of tuberculous caries, so that it was unlikely that the cord was being compressed by an uneroded intervertebral cartilage. It was decided to seek further information from myelography because of the uncertainty about the nature of the compressing lesion and its exact level in relation to the kyphosis.

Myelography. The opaque medium injected by lumbar puncture passed upwards to a complete obstruction at T6, just below the summit of the kyphosis, where it ended in a concavity suggesting the lower end of a tumour.

Operation. On 14.1.48 a laminectomy was performed from T1 to T8. There was no abnormality outside the dura mater. Opening the dura revealed an extensive mass of yellow fatty tissue which completely covered the upper part

INTRADURAL SPINAL LIPOMA

Fig. 1.—Right half of vertebral column, C2 to T10 inclusive, with cord and tumour *in situ.* The dura has been opened. The lipoma is within the pia mater and overlies the spinal cord posteriorly from C7 to T6. Gross kypho-scoliosis. Reduced.

PLATE LII

INTRADURAL SPINAL LIPOMA

FIG. 2 —Transverse section of pial lipoma situated on the posterior aspect of the spinal cord which is seen on the left, compressed and distorted. Heidenhain's iron hæmatoxylin. ×5.

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of the exposed spinal cord. There were no adhesions between the tumour and the dura, which appeared normal. The fatty tissue was presumed to be a lipoma and a piece for histology was cut out to a depth of 7 mm. without the spinal cord being seen. An attempt to separate the sharply defined lower margin of the tumour from the spinal cord had to be abandoned because they were too firmly adherent. The tumour appeared to lie beneath the pia mater and to be compressing the cord. The lower part of the exposed cord was normal.

After operation there was no change in the neurological condition and the patient died suddenly 36 hours later, presumably from the effect of œdema of the spinal cord.

Histology of tissue removed at operation

This consists of a mass of adipose tissue bearing a few blood vessels and delicate fibrous intersections. The free surface is covered by layers of areolar-type fibrous tissue, presumably the leptomeninges. There are no neural elements and no evidence of inflammation.

Post-mortem examination (16.1.48)

External appearances. The body was that of a heavily-built adult male with gross kypho-scoliosis of the upper thoracic spine. The scoliotic curve had caused displacement of the head and neck to the left side of the mid-line. Rotation of the mid-thoracic vertebræ had led to a prominent backward angulation of the ribs on the right side. There was a recent clean operation scar at the site of laminectomy in the upper thoracic spine.

Internal examination. *Trachea and bronchi* contained thick muco-purulent exudate. *Pleuræ.* Firm adhesions obliterated the right pleural cavity and were present also over the lower lobe of the left lung. *Lungs* œdematous and congested. There was an advanced degree of fusiform bronchiectasis affecting all lobes of both lungs, rigidity and thickening of the bronchial walls being more notable in the right lung than in the left. There was no naked-eye evidence of tuberculosis. *Pericardium* normal. *Heart* (13 oz.). Moderate increase of sub-epicardial fat. Dilatation of right auricle, myocardium pale, valves normal, coronary arteries healthy. *Aorta.* Early patchy atheroma, limited to the intima, was present, especially below the level of the renal arteries. *Other organs.* Apart from fibrosis of the capsule on the diaphragmatic surface of the spleen (3 oz.) there was no gross evidence of disease in the other viscera, including the brain.

Spine

The vertebral column from C2 to T10 inclusive was removed *en bloc* and subsequently sawn longitudinally, the plane of the saw-cut following the spinal curves in order to pass through the spinous processes and the mid-line of the vertebral bodies as accurately as possible. The right half of the specimen so obtained is shown in fig. 1.

The deformity. An exaggerated thoracic kypho-scoliosis extends from T1 to T8. The concavity of the backward arch is such that its limbs subtend an angle of 80° and there is a marked scoliotic convexity to the right, with rotation of the vertebral bodies. The deformity is corrected by a simple upward curve in the region C6 to T1. The rotated bodies of vertebræ T4-6 lie at the summit of the kyphosis, separated by distorted but otherwise healthy intervertebral discs. There is no evidence of tuberculous or other form of osteomyelitis.

The tumour. A banana-shaped fatty tumour extends from C7 to the lower border of T6 body, *i.e.* along the upper limb of the kyphosis. The dura mater is normal and not connected with the tumour. The tumour is fatty in appearance and overlies the spinal cord posteriorly from which it cannot be separated. Its surface is covered by a thin web of leptomeninges traversed by numerous delicate and congested blood vessels. The tumour appears to be entirely enclosed or embodied in the leptomeninges. It is nowhere adherent to the dura and it has no prolongations along the nerve roots.

Histology

A transverse block was cut of all the intradural structures—tumour and cord—at the level of the intervertebral disc between T1 and 2. Frozen and paraffin sections were prepared and stained with Sudan IV, hæmatoxylin and eosin, iron hæmatoxylin and van Gieson, and Heidenhain's iron hæmatoxylin (fig. 2). The tumour has the histological structure of a simple lipoma. It is composed of the adult form of adipose tissue cells, traversed here and there by nerve bundles and small well-formed blood vessels. A thin capsule of loose collagenous fibrous tissue invests the lipoma posteriorly and laterally. Anterolaterally, this fibrous capsule bifurcates to enclose the spinal cord, which is compressed and distorted but otherwise normal. It is clear, therefore, that the lipoma is embodied in the pia mater.

Ehni and Love noted connective tissue proliferation in association with pial lipoma which sometimes gave the appearance of invasion of neighbouring structures by the tumour. They record proliferation of the endoneurium and nerve roots traversing the lipoma, proliferation of the fibrous septum between tumour and cord, with extensions entering the substance of the cord, and thickening of the capsule of the tumour on its free surface, with adhesions forming between the pial mass and the dural arachnoid.

None of these features was present in our case, but although the tumour has so bland an appearance, its surgical removal at any stage must have been impossible in view of its intimate tissue connections with the spinal cord.

SUMMARY

A case is reported of intradural lipoma of the upper thoracic spine in a man of 40 with gross kypho-scoliosis. There was complete

paraplegia of recent development and there had been transient paraplegia twenty years previously. The tumour was a simple one of adult adipose tissue embodied in the pia mater.

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RETICULOSARCOMA OF THE THYROID GLAND

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(Plates LIII-LVIII)

In the earlier literature, sarcomata were frequently described as arising in the thyroid gland, but owing to inadequate description and illustration it is often impossible to decide whether sarcomatous or carcinomatous tumours were being considered. Later, Ewing (1940) expressed the opinion that all thyroid " sarcomata " previously reported were probably carcinomata which resembled sarcomata, and doubted whether any case of true sarcoma had ever been satisfactorily demonstrated. He pointed out that the " small-cell carcinoma " of the thyroid could readily be mistaken for sarcoma and placed the case of Jacobi and Bolker (1942) in this group. Wegelin (1926) took a less extreme view, believing that although many of the tumours described as sarcomata were of lepidic nature, true sarcoma did occur. Reticulosarcoma (Oberling, 1928) arising primarily in the thyroid gland must however be considered a rare disease.

Ambo (1937) described a " Retothelsarkom " of the thyroid and could find no other similar case in the literature. Rice (1932), however, gave a detailed account of five cases of "lymphosarcoma ", Dorothy Vaux (1937) reported a dictiocytic reticulosarcoma arising in a thyroid which contained large foci of lymphadenoid tissue, Graham (quoted by Joll, 1939-40) described a case of lymphosarcoma which he believed had developed from struma lymphomatosa, and Adé (1941) recorded a case of chronic atrophic thyroiditis developing into lymphosarcoma. Trempe et al. (1943) described a case of lymphoblastoma arising primarily in the thyroid, with secondary tumours in the testicle. These cases are discussed in greater detail later.

The present paper describes 5 cases of primary reticulosarcoma of the thyroid and discusses the relation of this neoplasm to the various types of lymphoid hyperplasia and in particular to the struma lymphomatosa of Hashimoto.

CASE REPORTS

Case I

A. M., a married woman aged 70, was admitted to the General Infirmary at Leeds under Dr John Towers on 5th April 1944, complaining of a swelling on the right side of the neck, first noticed in December 1943. It had increased

steadily in size but did not become painful or tender. For two months there had been hoarseness, dysphagia and slight dyspnœa on exertion, with some loss of weight. On examination a large swelling was found occupying the right anterior and posterior triangles of the neck and extending across the mid-line to the left side, where there was a small swelling deep to the sterno-mastoid muscle. The swellings were of " rubbery " consistency and irregular in outline ; they did not appear to be fixed to the skin or deeper structures and they moved on respiration. Radiological examination showed great cardiac enlargement and arteriosclerotic changes in the aorta. The swelling in the neck was considered to be a very large thyroid, without evidence of over-activity. The cardiac symptoms were thought to be due to myocardial fibrosis resulting from coronary atheroma and to be unassociated with the thyroid lesion. On 1st May 1944, bilateral thyroidectomy was performed by Mr Digby Chamberlain. The patient died 2½ years later, on 22.10.46 ; no further information is available.

The specimen removed at operation measured $4 \times 2\frac{3}{4} \times 2\frac{1}{4}$ inches in the vertical, transverse and anteroposterior diameters. On section it was irregularly lobulated, but nowhere presented an appearance suggesting thyroid tissue. Individual lobules were of tumorous aspect, the largest measuring 2½ inches in diameter. On section this tumour appeared to be malignant. Two smaller tumours, each about 1¼ inches in long diameter, were respectively intensely hæmorrhagic and very firm and white. A fourth smaller tumour about ¾ inch in diameter was also somewhat hæmorrhagic.

Histology

Three separate portions were taken for histology. Two of the tumours present are reticulum-cell sarcomata (fig. 1), being composed in the main of spheroidal and polygonal cells of fairly large size, often packed tightly together, but with an intervening network of reticulum (fig. 2). There are widespread early necrotic changes, and in these areas especially there are accumulations of lymphocytes and smaller numbers of polymorphs. There are also many large macrophage-type cells, some of them with foamy cytoplasm, others heavily charged with dark brown granular iron-reacting pigment. Even in the more cellular areas there are scattered lymphocytes, plasma cells and polymorphs. Mitotic figures are numerous in the tumour cells. Towards the periphery of these lobules, where they abut on broad fibrous septa, reticulum cells are fewer and have more abundant pink-staining cytoplasm, while lymphocytes are present in large numbers. In fact the appearance at the periphery is suggestive of a reticulosis rather than a reticulosarcoma. Many patches of hæmorrhage are associated with the necrobiotic change. A third main nodule consists in part of greatly altered thyroid or thyroid-adenomatous tissue, with a very abundant and intensely hyalinised stroma. Some of the acini contain rather pale colloid and are lined by cubical and flattened epithelium. Elsewhere there are smaller acini, both solid and luminated. Towards the periphery of this nodule there is an incomplete marginal zone of reticulosis or reticulosarcoma, with groups of small solid or luminated acini embedded either in the cellular tissue (fig. 3) or in densely hyalinised fibrous
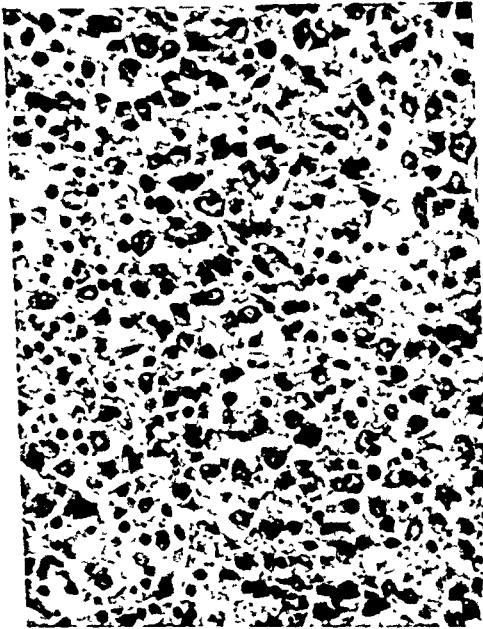
RETICULOSARCOMA OF THYROID

FIG. 1.—Reticulosarcoma, case 1. Hæmatoxylin and eosin. ×300.

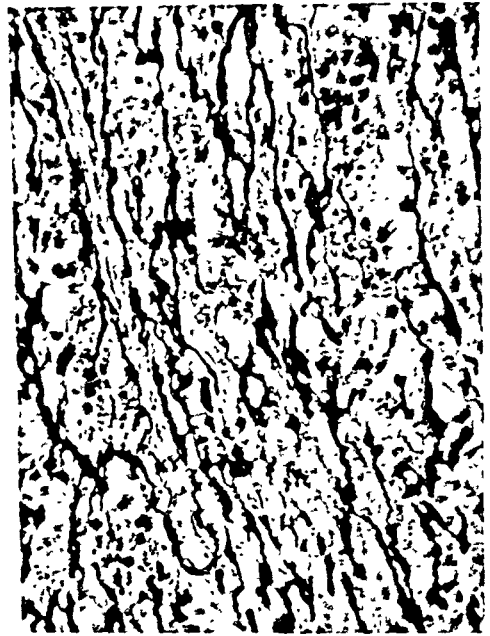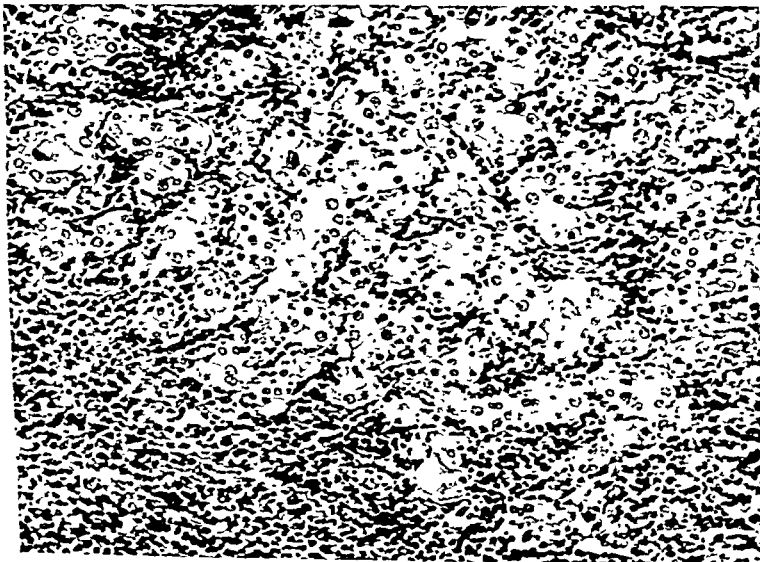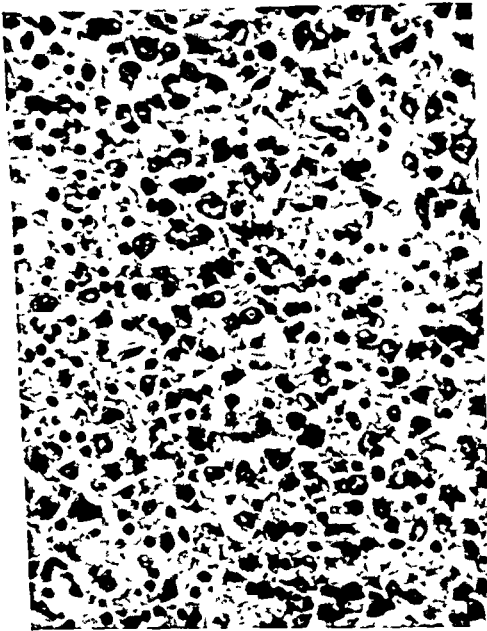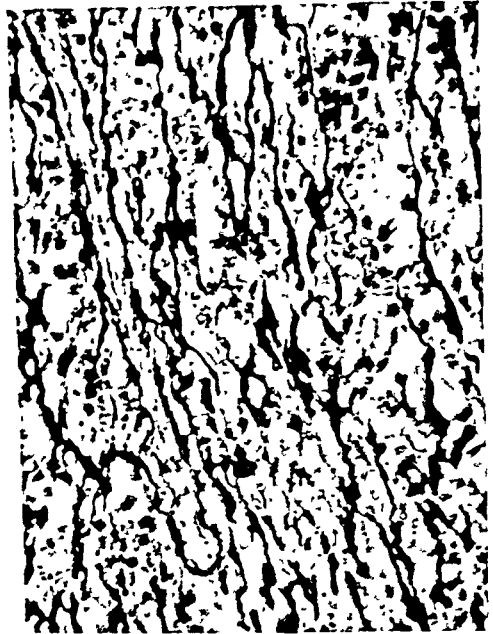
FIG. 2.—Reticulosarcoma, case 1. Silver impregnation. ×300.

FIG. 3.—Small thyroid acini embedded in cellular tissue mainly composed of lymphocytes. Case 1. H. and E. ×155.

steadily in size but did not become painful or tender. For two months there had been hoarseness, dysphagia and slight dyspnœa on exertion, with some loss of weight. On examination a large swelling was found occupying the right anterior and posterior triangles of the neck and extending across the mid-line to the left side, where there was a small swelling deep to the sterno-mastoid muscle. The swellings were of "rubbery" consistency and irregular in outline; they did not appear to be fixed to the skin or deeper structures and they moved on respiration. Radiological examination showed great cardiac enlargement and arteriosclerotic changes in the aorta. The swelling in the neck was considered to be a very large thyroid, without evidence of over-activity. The cardiac symptoms were thought to be due to myocardial fibrosis resulting from coronary atheroma and to be unassociated with the thyroid lesion. On 1st May 1944, bilateral thyroidectomy was performed by Mr Digby Chamberlain. The patient died 2½ years later, on 22.10.46; no further information is available.

The specimen removed at operation measured 4 × 2¾ × 2¼ inches in the vertical, transverse and anteroposterior diameters. On section it was irregularly lobulated, but nowhere presented an appearance suggesting thyroid tissue. Individual lobules were of tumorous aspect, the largest measuring 2½ inches in diameter. On section this tumour appeared to be malignant. Two smaller tumours, each about 1½ inches in long diameter, were respectively intensely hæmorrhagic and very firm and white. A fourth smaller tumour about ¾ inch in diameter was also somewhat hæmorrhagic.

Histology

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RETICULOSARCOMA OF THYROID

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FIG. 2.—Reticulosarcoma, case 1. Silver impregnation. ×300.

FIG. 3.—Small thyroid acini embedded in cellular tissue mainly composed of lymphocytes. Case 1. H. and E. ×155.

tissue. The arteries here show great medial hyalinisation and thickening of their walls, and sometimes intimal hyalinisation also.

Case 2

A. B., a married woman aged 76, had " for years " had a goitre which caused her no trouble. Six months before operation it began to increase rapidly in size and became painful. She also began to suffer from breathlessness, hoarseness and dysphagia, but without noticeable loss in weight. She was admitted to the General Infirmary at Leeds on 22.7.46, and on examination a hard tender swelling was found in the thyroid area, extending laterally behind the sterno-mastoid muscles. At operation on 30.7.46, Mr George Armitage found that the anterior part of the thyroid was very hard and adherent to surrounding structures. Seven-eighths partial thyroidectomy was carried out with some difficulty. The patient died three months later.

The excised thyroid was enlarged and its surface irregular, particularly that of the isthmus and right lobe. There were two " adenomata " in the left lobe : the isthmus had an appearance suggestive of carcinoma.

Histology

Sections from the apparently neoplastic tissue of the isthmus (fig. 4) show a characteristic reticulosarcoma composed of rounded or polygonal cells with ill-defined outlines and vesicular nuclei which are rounded or indented. Mitoses are numerous. A few nuclei are pyknotic. Occasional cells are binucleate. In one area the tumour is infiltrating adipose tissue (fig. 5) and voluntary muscle. Very sparse large pale cells suggestive of thyroid epithelium are also present. A second block, from the left lobe, includes the two " adenomatous " nodules of thyroid tissue above mentioned. These contain many moderately dilated thyroid acini lined by flattened or cuboidal epithelium. Most of the contained colloid is only faintly eosinophilic, but some of it in the smaller acini is strongly eosinophilic. The stroma is much hyalinised. There is diffuse infiltration of the surrounding loose fibrous tissue with lymphocytes, a few plasma cells and reticulum cells (fig. 6). Some of the thyroid epithelial cells are cuboidal or polyhedral ; a few are angular, elongated or wedge-shaped. Their cytoplasm is mostly pale-staining and finely granular, but there are occasional ill-defined hyaline areas in the larger cells. with a deeper eosinophilic tint suggestive of colloid. The nuclei vary in size and shape and some are hyperchromic. No mitoses are present. The epithelial cells of some acini have coalesced to form pseudo-giant cells. No lymphoid germinal centres can be identified. There is widespread hæmorrhage, but no vascular abnormality is apparent.

Case 3

S. P., a married woman aged 64, was admitted to the Royal Infirmary, Bradford, on 28th November 1934, complaining of a swelling of the front of the neck. This had first appeared six months previously and had gradually increased in size. It was associated with a choking feeling and breathlessness

on exertion, and she had recently had nausea, occasional difficulty in swallowing and some loss in weight. On examination there was some cyanosis of the face and neck. A large nodular swelling of dense consistency was present in the lower part of the neck, mainly on the left side and in front, with apparent extension behind the sternum. The trachea was displaced to the right and enlarged glands were palpable in both submaxillary regions. There were no symptoms of hyperthyroidism.

The patient was operated on by Mr F. W. Goyder on 29th November. The thyroid gland had a smooth surface and was of rubbery consistency. The left lobe was removed completely. It showed on section a uniformly white opaque appearance. A tracheotomy tube was inserted, and removed a month later when the patient was breathing freely. She was discharged in a satisfactory condition on 22nd June 1935.

On 28th August 1935, some 9 months after operation, she was readmitted to hospital under the care of Dr F. E. Chester-Williams, with large tumours in the neck and marked dyspnœa. Radium therapy was given on three occasions, but the patient died on 18th May 1936. No autopsy was obtained.

Histology

Only one block of tissue is available from this case, but it is unlikely from the description that any portion of the tumour showed frank thyroid tissue. The picture is mainly that of a homogeneous diffuse neoplasm, consisting of discrete cuboidal or rounded cells with relatively little cytoplasm (figs. 7 and 8). The nuclei are round or oval, with a rather fine chromatin structure, and contain one nucleolus or less commonly two prominent nucleoli. A few large binucleate cells are seen and mitoses are present in moderate number. The tumour cells suggest reticulum cells with a tendency to differentiation towards the lymphoblast. Silver impregnation shows that they are supported by a fine network of reticulin fibrils. Broad collagen septa are absent and there is no tendency to lobulation. There is no evidence of necrosis, hæmorrhage or hyaline degeneration.

Some parts show a few atrophic remnants of thyroid acini. These are usually compressed and devoid of colloid, but a few small round alveoli contain a small amount of eosinophilic colloid. The acinar epithelial cells are strongly eosinophilic and show marked variation in size of nuclei. In some cases the cells have coalesced to form multinucleated giant cells. No mitotic figures are seen. The tissue surrounding the atrophic acini consists mainly of small lymphocytes, but nowhere is there any suggestion of lymph follicles. Lymphocytes are also scattered among the tumour cells, and occasional single thyroid epithelial cells are recognised by their eosinophilic cytoplasm. Plasma cells are absent. No part of the section shows any well-formed thyroid structure.

Case 4

E. G., a married woman aged 63, was admitted to the Royal Infirmary, Bradford, on 20th October 1946, complaining of a feeling of pressure at the back of her throat from a lump which she had first noticed 6 weeks previously,
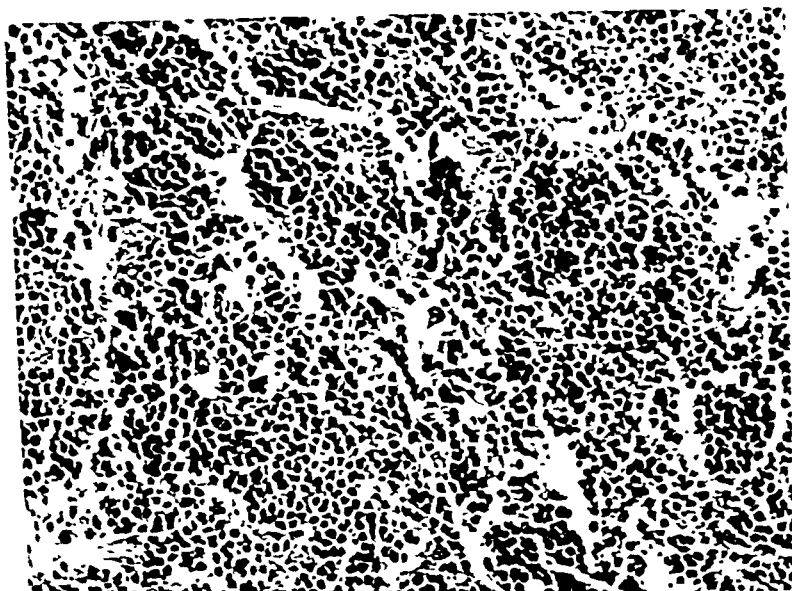
RETICULOSARCOMA OF THYROID
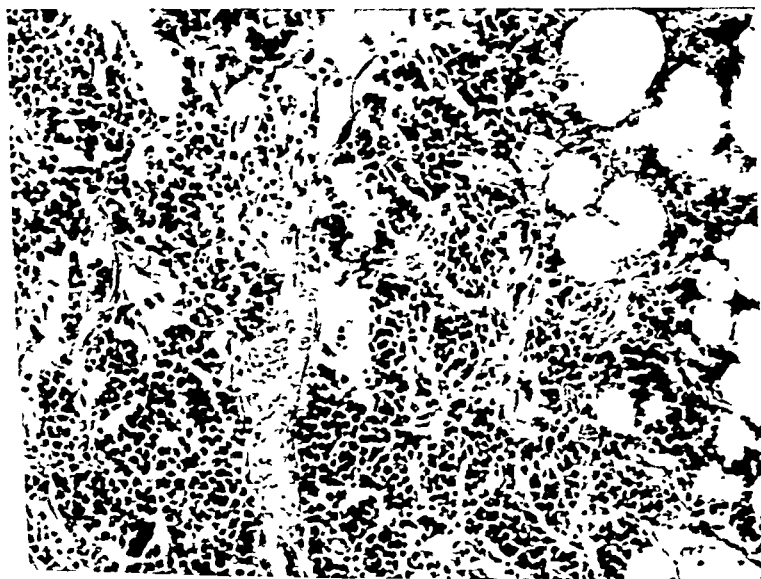

Fig. 4.—Reticulosarcoma, case 2.   H. and E.   ×155.

Fig. 5.—Reticulosarcoma invading adipose tissue.   Case 2.   H. and E.   ×155.

RETICULOSARCOMA OF THYROID
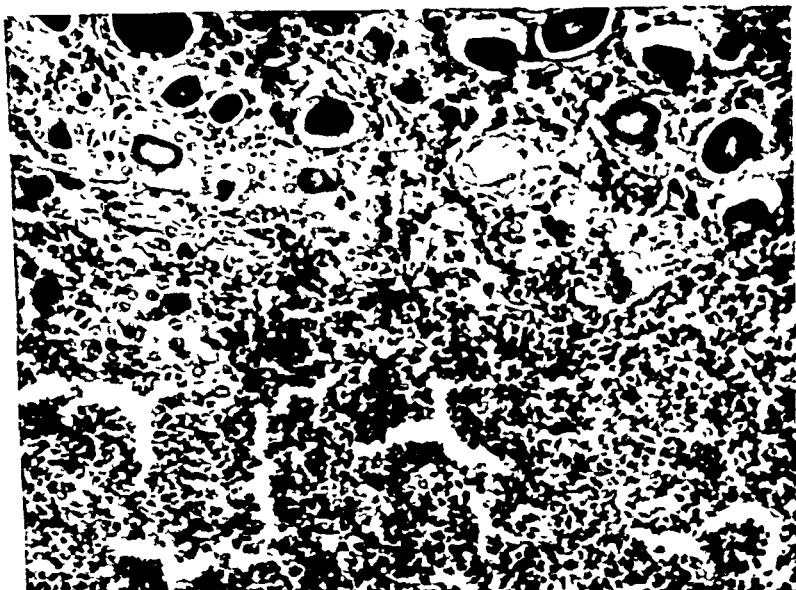

FIG. 6 —Altered thyroid acini related to a zone of infiltration with lymphocytes, plasma cells and reticulum cells. Case 2. H. and E. ×155.
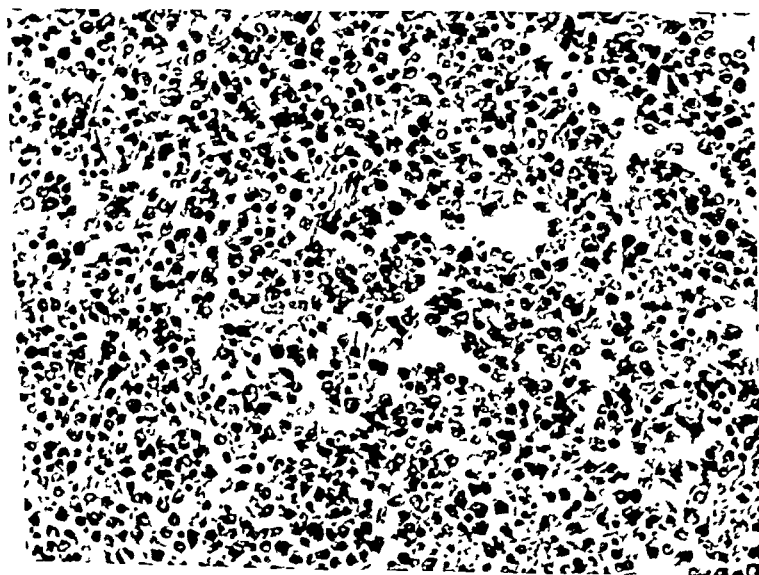

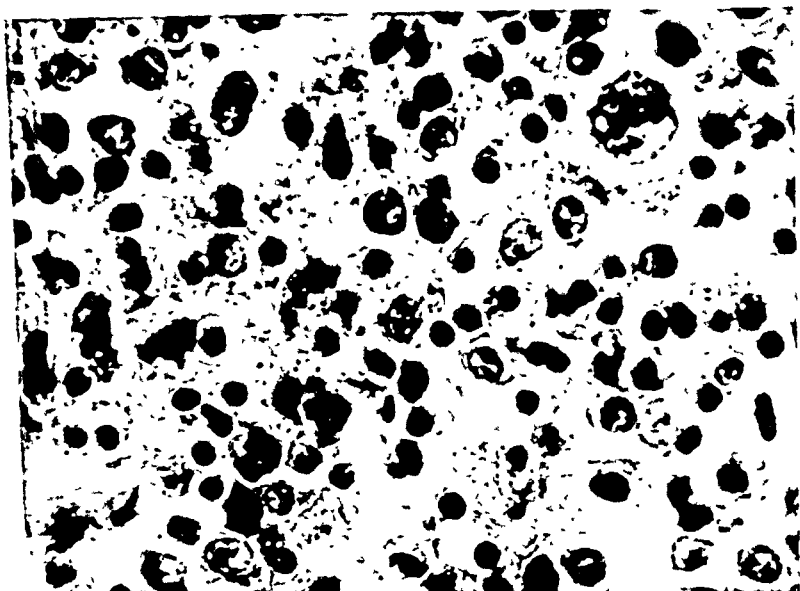FIG. 7.—Reticulosarcoma, case 3. H. and E. ×250.

RETICULOSARCOMA OF THYROID

FIG. 8.—Reticulosarcoma, case 3, showing, in the midst of the tumour cells, scattered large cells with small deeply-staining nuclei, apparently thyroid epithelial cells. H. and E. ×500.
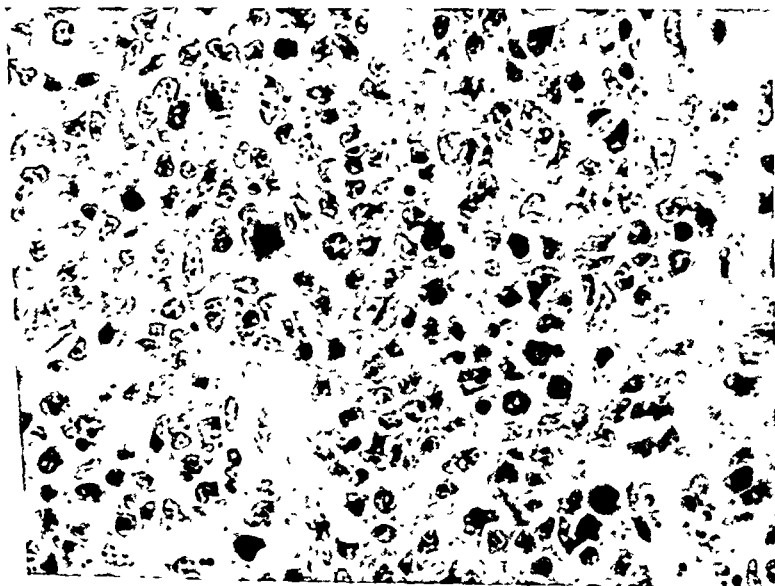
FIG. 9.—Reticulosarcoma, case 4. Mitoses are prominent in this field. H. and E. ×350.

RETICULOSARCOMA OF THYROID
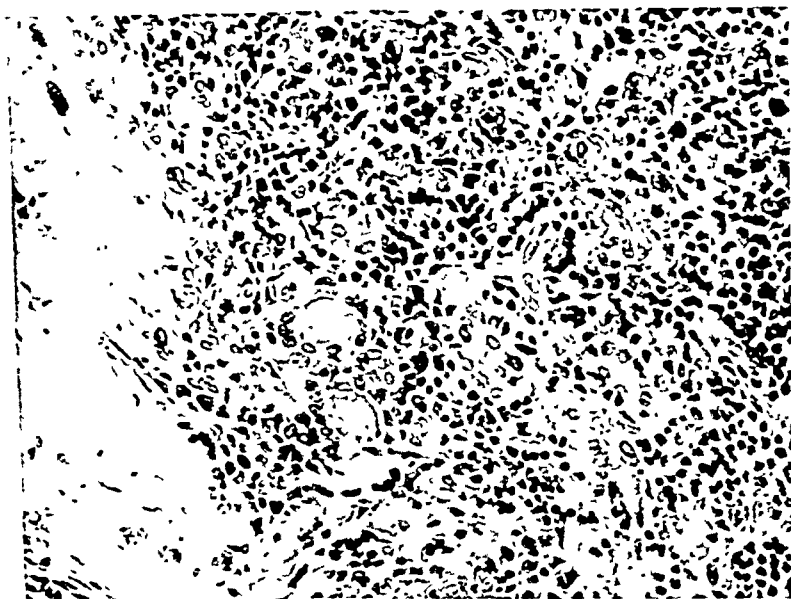
FIG. 10.—Thyroid tissue showing infiltration with lymphocytes and plasma cells. Case 4. H. and E. ×225.
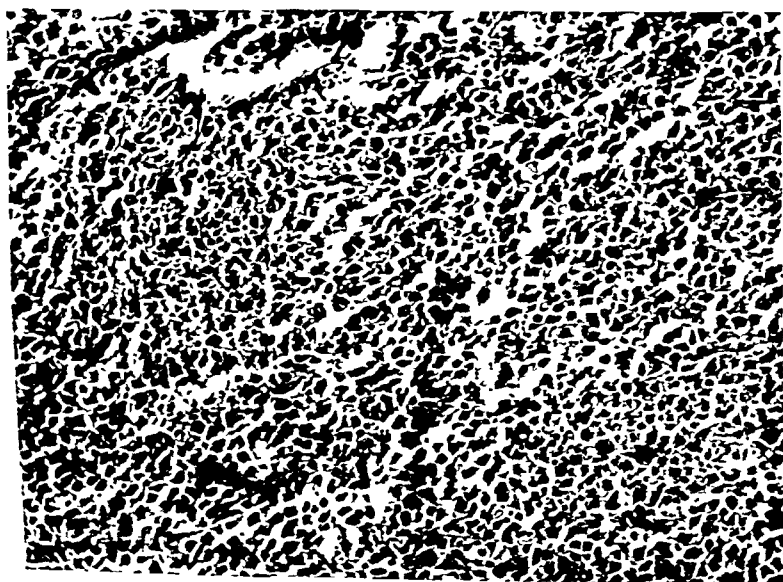
FIG. 11.—Reticulosarcoma, case 5. H. and E. ×155.

She had had slight difficulty in swallowing but no breathlessness. She had lost some weight recently. On examination a hard nodular tumour was felt in the right lobe of the thyroid, without fixation. There was no clinical evidence of hyperthyroidism and the patient's general condition was good. On 28th October the tumour was removed by Mr G. Whyte Watson, leaving only a small wedge of the right lobe posteriorly. The left lobe appeared normal. X-ray examination of the chest and skeleton showed no evidence of secondary deposits. The patient was transferred to the Regional Radium Institute, Bradford, on 28th November, under the care of Dr F. E. Chester-Williams, and was treated with radium to a total dosage of 4500r. When seen 18 months later the patient was well, with no signs of recurrence.

The excised tumour weighed 24 g. It was ovoid in shape, measuring 4·8 × 4 × 3 cm., and greyish-yellow in colour. The anterior surface was smooth, with a few remnants of fine adhesions. The upper third of the posterior surface was also smooth, but the lower two-thirds was rough and no capsule was present here. The cut surface was homogeneous and yellowish-white. The tumour was solid throughout and no tissue resembling thyroid tissue was found. The appearances suggested either neoplasm or lymphadenoid goitre.

Histology

The picture is that of a diffuse neoplastic process with a slightly lobular arrangement in places. Obvious thyroid tissue is present in one block only out of six taken from various parts. Most of the tumour exhibits a very great and very irregular reticulum-cell hyperplasia (fig. 9). The cells are polygonal, with ill-defined margins and intercellular processes. The nuclei are oval and often indented or lobulated, and the nucleoplasm is vesicular, containing one nucleolus and sometimes two nucleoli. Mitotic figures are frequent and there are occasional binucleate giant-cells. Nuclear pyknosis is prominent. A few lymphocytes and plasma cells are present even in the more homogeneous areas. The picture is further confused by the presence of scattered thyroid epithelial cells, appearing as clear cells with pale oval nuclei. Both small and large patches of necrosis are evident towards the centre of the tumour : these are fringed with lymphocytes, the nuclei of which show varying degrees of degeneration. Elsewhere, reticulum cells exhibit nuclear fragmentation. Congested blood-vessels and small haemorrhages are seen in certain parts, with traces of haematogenous pigment. A few collagen fibres traverse the tumour, and fibrous septa are present in the more heterogeneous areas. Reticulin staining reveals the presence of argyrophil fibres surrounding the remaining thyroid acini and forming a fine network among the proliferating reticulum cells. In many fields there lie scattered among the neoplastic cells a few large clear epithelial cells which occasionally form alveoli filled with eosinophilic colloid. Most of these cells have clear vacuolated cytoplasm and large clear vesicular oval nuclei, but a few have strongly acidophil cytoplasm. In one section there is obvious thyroid tissue consisting of acini of variable size lined by flattened or cuboidal epithelium and containing in the main poorly-stained and vacuolated colloid (fig. 10). Lymphocytes,

plasma cells, histiocytes and fibroblasts are abundant here, and there is a tendency to lobular arrangement, with peripheral collections of lymphocytes suggestive of hyperplastic lymph follicles. Many of these pseudo-follicles, however, show central necrosis with nuclear karyorrhexis. No areas of hyaline degeneration are found.

Case 5

A. W., a married woman aged 54, was admitted to the Duke of York Home, Bradford, suffering from a swelling of the neck of four years' duration. This had increased rapidly in size during the previous four months, and for four weeks it had been causing dyspnœa and hoarseness. There was no loss of weight. On examination there was smooth elastic enlargement of the thyroid gland, which was tense within its capsule. The patient was rather obese, with a myxœdematous facies, but the pulse rate was normal. Partial resection of the thyroid was carried out by Mr Peter McEwan on 13.8.44, with the object of relieving pressure symptoms. On 1.9.44 the patient was transferred to the Regional Radium Institute, Bradford, under the care of Dr F. E. Chester-Williams. The thyroid was found to be still enlarged and the circumference of the neck at the level of the operation scar was 43 cm. She was given deep X-ray therapy, 800r to each of four fields of the neck. This caused relief of symptoms and rapid decrease in the size of the tumour. After discharge the tumour recurred and the patient died one month later (7.10.44).

The operation specimen consisted of several large masses of soft, fairly uniform tissue, with a few opaque patches. The appearances suggested a malignant neoplasm.

Histology

Six blocks taken from various parts of the specimen show a diffuse malignant growth with no frank thyroid tissue, though acinar remnants are present in all sections. The tumour is a pleomorphic reticulo-sarcoma with both undifferentiated and differentiated areas (fig. 11). In the former the tissue is composed mainly of rather large cuboidal or polygonal cells united by intercellular processes. These cells have one or two oval, indented or lobulated nuclei with vesicular nucleoplasm containing one or more eosinophilic nucleoli. Mitotic figures and multinucleated giant-cells are present in small numbers (fig. 12). The cells are supported by a delicate network of reticulin, well shown by silver impregnation (fig. 13). Dense collagenous bands are absent. There are a few scattered lymphocytes but no plasma cells. A few isolated thyroid epithelial cells with strongly eosinophilic cytoplasm and small clear nuclei are present. The differentiated areas show many smaller discrete cuboidal or round cells with scanty cytoplasm and more rounded nuclei. Mitoses are frequent, and occasional binucleate cells occur. Many of the nuclei are pyknotic. Silver staining reveals many fine reticulum fibrils. A few lymphocytes but no plasma cells are seen. Many scattered acidophilic thyroid epithelial cells are present and there are occasional thyroid acini, in some of which the cytoplasm is eosinophilic, in others vacuolated and poorly stained. Some sections show small areas of hæmorrhage
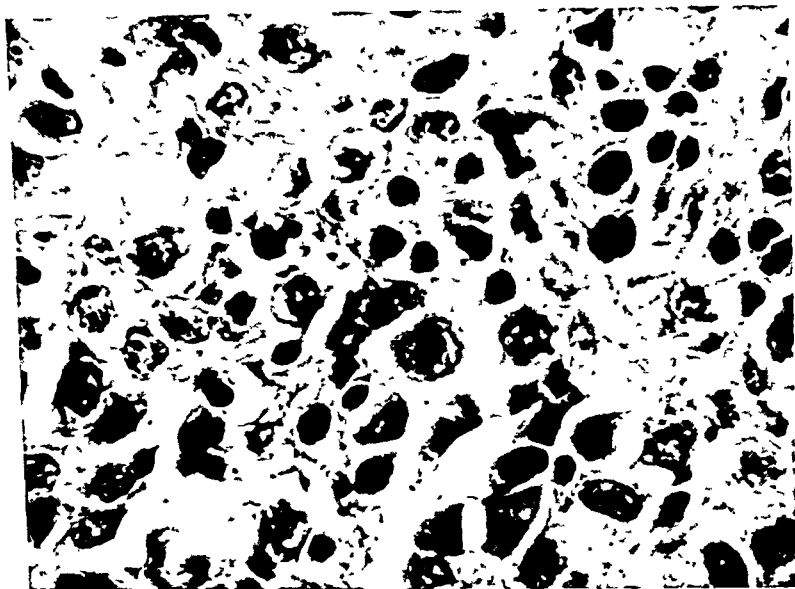
RETICULOSARCOMA OF THYROID

FIG. 12.—Reticulosarcoma, case 5, showing mitoses. H. and E. ×500.

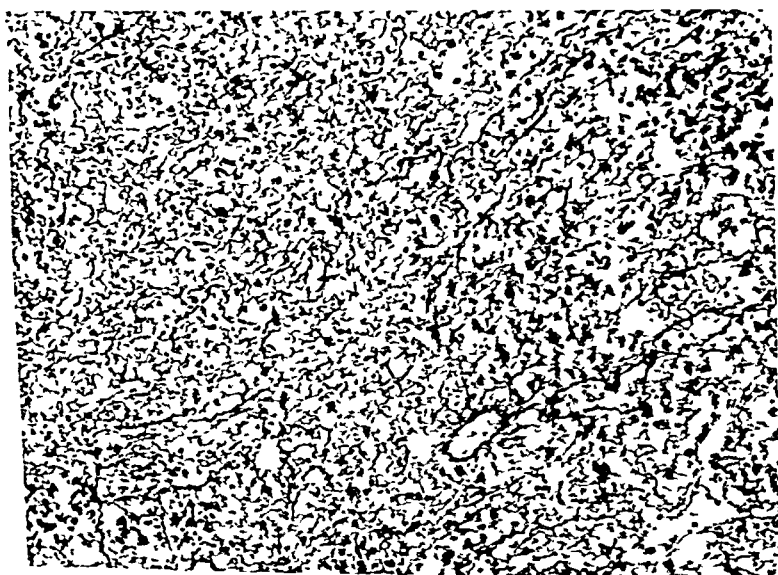FIG. 13.—Reticulosarcoma, case 5. Silver impregnation. ×155.

and necrosis, and in one block striated muscle is invaded by the tumour cells. The arteries appear normal.

COMMENTARY

The five cases of reticulosarcoma here described were all in elderly women in whom the thyroid gland had increased rapidly in size during the previous few months. In two cases a goitre had pre-existed. All the patients complained of pressure symptoms, but there was no clear evidence of either hypo- or hyperthyroidism. Three of the tumours were focal and nodular, the other two diffuse and adherent to surrounding structures.

Four of the patients died within two years of operation and in two of these there was clinical evidence of malignant recurrence. The fifth (case 4), who had received early intensive radium therapy after operation, is alive and well after two years.

Histological examination of the tumours removed at operation showed a picture of diffuse reticulosarcoma. In cases 1 and 4 the growths were relatively undifferentiated : case 5 showed undifferentiated areas. More or less advanced necrosis of tumour tissue was present in four, all showed groups of atrophic thyroid acini scattered throughout the neoplasm and tumour-free thyroid tissue was present in 3 cases.

The differential diagnosis of these tumours has to be made from anaplastic carcinoma on the one hand and lymphadenoid goitre on the other. Ewing doubted the occurrence of a true sarcoma of the thyroid gland, but both Wegelin and Rice were satisfied as to its existence. It is, however, a rare tumour. In none of our cases was the histology suggestive of carcinoma. Islets of altered thyroid acini and scattered epithelial cells sometimes required careful differentiation from the surrounding tumour cells, but these epithelial foci were of an atrophic nature and showed no transition to the malignant cells. Reticulosarcoma, unlike anaplastic carcinoma of the thyroid, is very sensitive to radiation (Haagensen. 1931). There is clear evidence of this sensitivity in case 5, but in case 3 the data available are insufficient to allow us to be certain of this.

Williamson and Pearse (1925) have emphasised the difficulty of distinguishing between sarcoma and lymphadenoid goitre. Sections from cases of struma lymphomatosa examined by us have shown a similar histological picture in some high-power fields, but this was always limited to centres of follicles and the follicular structure was not destroyed. It is possible that Ewing's photomicrograph was taken from such a germinal centre and may be misleading. A similar appearance was displayed in some of the present series of cases, but was distributed diffusely throughout the tumour. Our cases showed notable proliferation of reticulum cells, with numerous mitoses; fibrous tissue was generally scanty. Only in case 4 was there a tendency

to follicular arrangement in places and here it was associated with central necrosis.   On the other hand the uninvolved thyroid tissue in some of the tumours had similar histological features to those of struma lymphomatosa (vide infra).

Histogenesis

Ehrlich and Gerber (1935), discussing the histogenesis of lymphosarcomatosis, state that although the reticulum cell is universally present in the human body, it is the reticulum cell of lymphatic tissue which is predominantly involved in the neoplastic process.   It would be reasonable to assume, therefore, that the thyroid, which is often the seat of lymphoid hyperplasia, might well be particularly prone to tumours of this group.   The most striking lymphoid hyperplasia in the thyroid occurs in struma lymphomatosa, described by Hashimoto in 1912, but it also occurs in struma fibrosa.   Foot, in a personal communication to Poer et al. (1936), expresses the belief that the lymphocytes in struma fibrosa are not so frequently associated with germinal centres as they are in struma lymphomatosa.   The difference or identity of these two conditions is still in dispute.   In their most fully developed form they appear as distinct pathological entities, but it must be admitted that there are many intermediate forms which partake of the characters of both.   These characters must now be considered in a little detail, since they are of importance in determining whether or not they preceded the onset of reticulosarcoma in the present cases.

Most of the American observers have considered the two conditions to be distinct (Foot, quoted by Poer et al., 1936; McClintock and Wright, 1937; Hellwig, 1938; Schilling, 1945).   Joll (1939–40) also held this view.   Those who believe the two conditions to be stages of one process include Vaux (1938), Renton et al. (1938–39) and Ewing (1940).   For the purpose of the present study, struma lymphomatosa and struma fibrosa will be considered as separate entities.

According to Schilling, the thyroid of struma lymphomatosa is diffusely enlarged and its surface pseudo-lobulated.   On incision, it is finely lobular and slightly yellowish.   Joll states that neither necrosis, calcification nor abscess formation was observed in any specimen examined by him.   Struma lymphomatosa has three main histological characters : lymphoid hyperplasia, epithelial changes and increase in the fibrous stroma.   The lymphoid hyperplasia has been described by Joll as so diffuse and widespread that the histological appearances in all parts of the gland are similar.   Schilling also states that there is a diffuse infiltration by lymphocytes throughout the gland.   Lymph follicles with germinal centres are characteristically present.   Plasma cells are abundant and Renton et al., adduce this as evidence of an inflammatory process.

Changes in the thyroid epithelium are considerable.   Muir (1936) is of the opinion that the acinar changes are primary and the lymphocytic infiltration secondary, as in exophthalmic goitre.   Parmley and Hellwig (1946) also consider that the acinar changes are primary.   They may certainly resemble those seen in the lymphoid hyperplasia of Graves's disease, as emphasised by Graham and McCullagh (1931), and Goldberg and Davson (1948–49) believe that no clear distinction can be made between toxic goitre with lymphoid infiltration and lymphadenoid goitre ; the difference is one of degree.   All this would suggest

that the histological recognition of early struma lymphomatosa is fraught with great difficulty. The epithelial changes consist essentially in degeneration of the acini with shrinkage and coalescence of the epithelial cells (Schilling). This gives rise in some cases to pseudo-giant cells. The acini tend to be smallest where the lymphoid hyperplasia is greatest; here they are often devoid of colloid and may be solid. The epithelial cells themselves change, assuming a large cuboidal or polyhedral form, with nuclei which are usually centrally placed, hyperchromic and variable in size. Sometimes these cells are strikingly eosinophilic and liver-like (McClintock and Wright). Hellwig has described similar if not identical eosinophilic cells, some of which were intercalated with those of normal appearance in the acini. Sometimes solid strands of these cells are present. Reist (quoted by Hellwig) believes that such cells are a sign of degeneration. Parmley and Hellwig describe apparently identical cells as arranged either in acini or solid strands surrounded with lymphoid tissue: they believed them to be " protoplasm-rich " cells of Hurthle. Eosinophilic cells of this kind occur in diverse conditions of the thyroid : they have been observed by us in a classical case of struma fibrosa.

Hashimoto himself regarded an increase in the fibrous stroma as a character-istic of struma lymphomatosa. Schilling described the fibrous overgrowth as having a more delicate texture than the densely collagenous type found in struma fibrosa and Hellwig described four cases in which there was no increase.

The gross changes in struma fibrosa are well known. They include both the " iron-hard " consistency and adhesion to surrounding structures. Histologically there is great overgrowth of the fibrous stroma, which is highly collagenous and forms wide hyalinised bands. Finer collagenous strands pass between the individual acini—an appearance not seen in the advanced form of struma lymphomatosa. As Foot has stated (Poer) " the fibrous overgrowth seems to strangle the parenchyma slowly but certainly," in contrast to struma lympho-matosa, where " we have the lymphoid tissue growing as lymph follicles among the parenchymatous portion of the gland, and crowding it out that way." Nevertheless Hashimoto, in his original description of struma lymphomatosa, described sparse acini " distinguished by their small size " and lying in the hyperplastic fibrous stroma.

Schilling states that in both struma fibrosa and the condition he describes as pseudo-giant-cell thyroiditis (apparently a variant of the other) there is thickening of both intima and media of the arterioles. He mentions that German has described this condition and has demonstrated it by means of a del-Rio Hortega silver method. This vascular change he regards as a point of distinction between struma fibrosa and struma lymphomatosa. Goldberg and Davson found that calcification or intimal sclerosis occurred in the larger arteries of the thyroid gland in the age-groups in which lymphadenoid goitre is common, but seemed to bear no relationship to the lymphadenoid changes themselves.

Lymphoid hyperplasia is a well-known characteristic of thyrotoxic goitre. It also occurs in the atrophic thyroid of myxoedema and in conditions of hyper-involution. In these conditions it is essentially patchy and never generalised or diffuse, as in struma lymphomatosa (Joll). Even the normal thyroid may show lymphoid follicles. Simmonds (1913) has described accumulations of lymphocytes in thyroids which appeared macroscopically normal. They were rare before puberty, became more common with advancing age and were commoner in females. Nolan (1938) found " lymphocytic foci " in 18·4 per cent. of thyroids obtained at routine autopsies. They were somewhat more common in females and bore no apparent relation to any particular type of disease, apart from exophthalmic goitre. Joll figures a lymphoid follicle with a germinal centre of Flemming type from a normal thyroid. Smith (1930) states that of normal thyroids removed at autopsy in subjects who had died from disease not involving this gland, 15 per cent. showed lymphoid hyperplasia.

Finally it should be remembered that reticulosarcoma of the thyroid may be a secondary condition due to direct spread or blood-borne metastasis from some other (primary) focus. Willis (1931) cites a case of endothelioma of the cervical lymph-nodes invading the thyroid described by Flournoy in 1907. Ghon and Roman (1916) describe a series of 27 cases of reticulosarcoma in which secondary thyroid involvement occurred in one case by direct extension and in two by blood-borne spread. In one case the acini were atrophic and some had completely disappeared. Ehrlich and Gerber (1935), in a series of 18 cases of lymphosarcomatosis, found three in which there was thyroid involvement; the acinar epithelium showed no significant change. A case is also mentioned by Mayo and Schlicke (1941).

Relation of reticulosarcoma to pre-existing lymphoid hyperplasia

The five cases of reticulosarcoma of the thyroid here recorded and certain related cases in the literature will now be discussed from the point of view of their possible origin in a pre-existing lymphoid hyperplasia, particularly struma lymphomatosa.

In only three of the present series (cases 1, 2 and 4) is tumour-free thyroid tissue available for study. Undoubted lymphoid hyperplasia is present in these thyroids, but it differs in two respects from that of struma lymphomatosa : the distribution is less generalised, many low-power fields of cases 1 and 2 being wholly devoid of lymphocytes, and germinal centres are absent, except in case 4, where the follicles are atypical and show some degree of central necrosis. Plasma cells are also present in cases 1 and 4 but there is little intermingling with the tumour cells.

Acinar changes are pronounced and resemble those seen in struma lymphomatosa. In sparse areas the acini are small, devoid of colloid and sometimes solid ; they are composed of large cells, sometimes cuboidal, sometimes polygonal, with pink granular cytoplasm and the occasional suggestion of intracellular colloid. The nuclei show great variation in size, and possess as a rule only one nucleolus, although as many as five have been seen. More frequently the acini are luminated and lined by flattened cells, or by clear cells with weakly-stained or vacuolated cytoplasm. Moreover, adenomata are present in cases 1 and 2 and show degenerate areas with a pale hyaline matrix in which the infrequent acini lie singly or in small widely-separated groups : these were not found in Joll's series.

In two of the cases there is overgrowth of fibrous tissue and a few dense collagenous bands are present, but this has a limited distribution and there are no fine strands passing among the acini and "strangling" them as in struma fibrosa. Thickening and hyalinisation of the media and intima of the arteries have been described in struma fibrosa and its giant-cell variant, but are absent in struma lymphomatosa (Schilling). They are present in case 1 of this series, but the clinical history suggests that this may be part of a general arteriosclerosis and merely incidental.

Although the possibility cannot be entirely excluded that these

tumours arose from early pre-existing struma lymphomatosa, it seems more probable that they developed in the lymphoid hyperplasia which is so often found in the thyroid of elderly female subjects.

Rice described five cases of lymphosarcoma of the thyroid in detail ; again all the patients were women aged 50-80. In each, the thyroid tissue not involved in the tumour was described separately, and was found to show lymphoid hyperplasia. In two the lymphoid tissue was scanty, and in these the possibility of a pre-existing struma lymphomatosa can be excluded. In the other three, lymph follicles with germinal centres were present. Rice believed that his observations gave no support to the theory that chronic thyroiditis could develop into lymphosarcoma, although the possibility could not be excluded. It is clear that he considered both struma fibrosa and struma lymphomatosa to be variants of a chronic thyroiditis. He stated that plasma cells and fibrous overgrowth were absent in his cases, and adduced this as part of the evidence in establishing them as cases of the true lymphosarcoma rather than chronic thyroiditis. He believed, however, that the tumour had originated from lymphoid tissue in the thyroid.

Adé described a lymphosarcoma of thyroid in a woman of 70. The gland was the seat of chronic atrophic thyroiditis with lymphocytic infiltration : myxœdema had existed before the onset of the tumour. The lobe of the thyroid not involved in the growth showed considerable infiltration with lymphocytes and plasma cells, separating and destroying the vesicles.

Trempe *et al.* described a case of lymphoblastoma arising in the thyroid of a man aged 60 who had had goitre for eleven years. The further enlargement had begun a few months before admission. The gland was extensively calcified. The testicles were the seat of tumours which had the same histological characters as the thyroid growth. The authors believed that the lymphoblastoma had arisen in an old goitre with calcification, although they could not prove this. The histological characters of the thyroid were not described, but the calcification would exclude a previous struma lymphomatosa if Joll's criteria are accepted. This is the only case of reticulosarcoma of the thyroid in a male among all the cases described or cited in this paper.

CONCLUSION

Struma lymphomatosa is not a well-defined entity, and after reviewing the literature we are not prepared to express a definite opinion on the various points at issue. If, however, the views of Joll and of Schilling are accepted, it becomes clear that the entity they describe as struma lymphomatosa has not yet been identified with certainty as the precursor of reticulosarcoma or lymphosarcoma of the thyroid. Such a neoplasm certainly arises from lymphoid tissue in the gland, but such tissue may exist in a variety of conditions and indeed its presence is fairly common. It would appear that reticulosarcoma may take origin in any of these types of lymphoid hyperplasia in the thyroid, and it is, perhaps, the relative rarity of both reticulosarcoma and struma lymphomatosa that has caused their certain association to remain unrecorded.

Five new cases of primary reticulosarcoma of the thyroid gland are described, all of them in elderly women.

We wish to thank our hospital colleagues in Bradford and Leeds for their co-operation and for permission to use their clinical records. We are specially indebted to Professor Matthew J. Stewart and Dr C. J. Young for their encouragement and advice, and for their courtesy in providing records of case 1 and cases 3 and 5 respectively.

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THE PRESENCE AND SIGNIFICANCE OF ALKALINE PHOSPHATASE IN THE CYTOPLASM OF MAST CELLS

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(PLATE LIX)

THE behaviour of cells in the course of wound healing is " strikingly similar to that of the cells of a fragment of tissue cultivated *in vitro* " (Ludford, 1929, p. 194). Nevertheless the factors which regulate cellular proliferation *in vivo* remain obscure.

According to Sylvén (1941) reparative hyperplasia in the skin of the rat is initiated by the release into the tissues of a metachromatically-staining substance (free chromotrope substance—F.C.S.) which is derived from the granules of histogenous mast cells. As a·result of his studies on tumour growth, Sylvén (1945, p. 13) believes that the function of this F.C.S. is to provide for the growing cells a matrix which " resembles the nutritional media used in tissue cultivation."

As wound repair in the rat approaches completion and as the F.C.S. disappears (Sylvén, 1941), Fell and Danielli (1943) observed that the enzyme alkaline phosphatase participates in the laying down by fibroblasts of the argyrophil fibrils which are believed to be the precursors of fresh collagen (Maximow, 1927-28 ; Wolbach, 1933 ; Hass and McDonald, 1940 ; Wolfe and Wright, 1942). Like the F.C.S. of the earlier stages of wound healing the alkaline phosphatase is distributed diffusely throughout the growing zone.

In view of Sylvén's contention that F.C.S. in the rat is derived from the granules of histogenous mast cells, it was of particular interest to note that Noback and Montagna (1946) had recently demonstrated alkaline phosphatase in the granules of mast cells in, this animal. It was therefore decided to investigate the possibility that alkaline phosphatase might be present in the granules of mast cells of other species and that, like F.C.S., it might be released into the tissues to play a part in the regulation of cell growth. On theoretical grounds the simultaneous presence of F.C.S. and alkaline phosphatase in the same cell is not incompatible with the ability of these agents to function in sequence, since the acidity of a substance exhibiting metachromasia (Lison, 1935) would effectively inhibit an enzyme

requiring for its optimum activity an alkaline pH around 9·4. Only when the F.C.S. had ceased to stain metachromatically would the enzyme become active.

Accordingly it was decided (1) to examine the mast cells in a variety of animal species for alkaline phosphatase by means of the technique devised by Gomori (1939), and (2) to determine whether any metachromatically-staining F.C.S. which was encountered also stained positively for this enzyme. A further purpose of the present investigation was to seek fresh evidence concerning the origin of histogenous mast cells since, up to the present, only tentative conclusions have been drawn (Michels, 1938; Cramer and Simpson, 1944; Holmgren, 1946-47).

MATERIALS AND METHODS

Tissue sections from the following species were examined for the presence and distribution of (1) mast cells, (2) free chromotrope substance, and (3) alkaline phosphatase : man, mouse, rat, cat, dog, horse, rabbit, guinea-pig, mole, fowl, frog, axolotl and brown trout. Tongue and skin were selected as being likely to contain the maximum number of mast cells (Williams, 1900). Other tissues rich in mast cells which were also examined included intestine of fish (Michels, 1923), a biopsy specimen from a case of urticaria pigmentosa (Unna, 1896) and the skin of mice treated with methyl cholanthrene in benzene (Cramer and Simpson).

Fixation and embedding

A preliminary trial of 4 per cent. basic lead acetate as a separate fixative for the demonstration of F.C.S. (Holmgren, 1940) proved unsatisfactory owing to the rapid deterioration of this solution. The following technique was therefore adopted for all specimens.

Fresh tissue blocks cut with a sharp safety razor blade to a thickness of 2-3 mm. were fixed in 80 per cent. alcohol for 12 hours and subsequently passed through absolute alcohol (12 hours), chloroform (two changes of 3 hours each), chloroform and paraffin equal parts (half an hour in the paraffin oven at 57° C.) and finally embedded in paraffin in a vacuum bath (6 hours at 57° C.). Sections cut at 2 μ.

Staining

(1) The characteristic metachromatic tint of the mast-cell granules and F.C.S. was demonstrated by staining sections for 30 minutes in a 1 per cent. solution of toluidine blue in 1 : 40 alcohol (Sylvén, 1941), after which excess stain was removed with absolute alcohol and the sections were cleared in xylol and mounted in neutral Canada balsam.

(2) Gomori's method (1939, 1941) as modified by Kabat and Furth (1941) was used to indicate the presence of alkaline phosphatase. This method relies upon the ability of alkaline phosphatase to liberate phosphate from an ester linkage. In the substrate, sodium β-glycerophosphate provides the phosphate ions which, after liberation by alkaline phosphatase, combine with calcium from added calcium nitrate to form the relatively insoluble calcium phosphate. The calcium phosphate so produced remains at the site of its formation and therefore has the distribution of the enzyme itself. The substrate also contains magnesium sulphate, since magnesium ions have been found to potentiate alkaline phosphatase activity (Erdtman, 1927), together with sodium barbitone

to act as a buffer for the maintenance of the alkaline reaction (pH 9·4) at which the enzyme is most active. The final step in the procedure consists in rendering the sites of enzyme activity visible by converting the calcium phosphate— through cobalt phosphate—to the black insoluble cobalt sulphide.

The practical application of the technique was as follows :—

(1) The sections after clearing in xylol and removal of xylol by rinsing in absolute alcohol were left for 12 hours at 37° C. in the following solution :—

3·2 per cent. sodium β-glycerophosphate .		6 ml.
2·0 „ calcium nitrate . . .		9 „
10·0 „ sodium barbitone . .		6 „
0·01 M magnesium sulphate . . .		6 „
Distilled water		23 „

(2) After removal from the substrate the sections were :—

    (a) thoroughly washed with 1 per cent. calcium nitrate,
    (b) covered with 2 per cent. cobalt nitrate (2 minutes),
    (c) thoroughly washed with distilled water,
    (d) covered with ammonium sulphide (1 ml. fresh ammonium sulphide to a Coplin jar of distilled water),
    (e) washed in tap water,
    (f) dehydrated rapidly through the alcohols,
    (g) cleared in xylol,
    (h) mounted in neutral Canada balsam.

The individual stages outlined above comply with the recommendations made by Danielli (1946) in his critical review of Gomori's method except for the employment of vacuum embedding. This procedure causes a slight loss of enzyme which, however, is uniform throughout the section.

(3) In addition the following procedures were carried out for each specimen :—

    (a) As a control to the foregoing technique, the presence of pre-formed phosphate was excluded by the use of a substrate from which the phosphoric ester had been omitted.
    (b) A duplicate set of Gomori-treated sections was counterstained with toluidine blue to show up mast cells which had not been stained by the Gomori reagents.
    (c) A section of each tissue was stained with hæmatoxylin and eosin for the verification of topographical detail.

Results

1. Alkaline phosphatase in mast cell granules

In the tissues of the mouse and rat, alkaline phosphatase was consistently demonstrated in a proportion of the mast cells and appeared to have the same form and distribution in the cytoplasm as the normal metachromatic granules of those cells (fig. 1). The phosphatase-containing mast cells were situated mainly near capillaries, but even in these areas there were many mast cells which lacked the enzyme either wholly or in part. In contrast to the fully positive cells, which were often so crowded with stained granules as to appear almost uniformly black, others contained only a few granules ranging in colour from pale to dark brown. The simultaneous presence in

the mast cell of metachromatic material as well as alkaline phosphatase was investigated by superimposing toluidine blue on Gomori-treated sections (fig. 2). This modification of technique showed that a single mast cell might contain in its cytoplasm a variety of granules some of which gave a strong metachromatic tint with toluidine blue while others stained black when treated by the Gomori method. Granules which reacted weakly to the Gomori reagents proved capable of further staining in a metachromatic tint when the counter-stain was applied, but such metachromatic staining was never observed in granules in which the Gomori reaction had initially been intense. This technique gave such clear-cut results that it was employed again for the investigation of phosphatase activity in species in which mast cells are scanty. Gomori-treated sections were re-examined immediately after counterstaining with toluidine blue so that any discrepancy in the numbers of mast cells revealed by the two methods could be readily detected and a decision reached as to the number of mast cells which had stained by Gomori's method. Even with this degree of control some doubt remained concerning the presence of alkaline phosphatase in the mast cells of the skin of the dog and guinea-pig. The enzyme was considered to be absent from the mast-cell granules of all the remaining species examined. This included man, since even the numerous mast cells in a case of urticaria pigmentosa were all negative to Gomori's test.

2. Free chromotrope substance

Patches of metachromatically-staining material, ranging in colour from violet to red, were found in most of the sections examined and commonly occurred at sites where active growth was in progress, as in hair follicles and the loose connective tissue immediately beneath the epidermis. It proved impossible, however, to demonstrate the simultaneous presence of alkaline phosphatase in these areas, with the possible exception of the cock's comb, the intestinal wall of the trout and the stroma of mouse papillomas. Even in these areas it was suspected that the association of the two factors was fortuitous. Thus in the highly vascular tissue of the cock's comb and the stroma of mouse papillomas the alkaline phosphatase could equally well have been derived from damaged vascular endothelium, which in these two species is rich in the enzyme (Moog, 1944; Biesele and Biesele, 1944). Similar artefacts could conceivably account for the mingling of F.C.S. and alkaline phosphatase in the intestinal wall of the trout, in which the mucosal cells are rich in the enzyme and the mast cells are excessively fragile, readily parting with their meta-chromatic material (Michels, 1923). Although the addition of mercuric chloride to the alcohol greatly improves the fixation of the granules in the fish (Duthie, 1938-39), this cannot be employed preparatory to the use of Gomori's method.
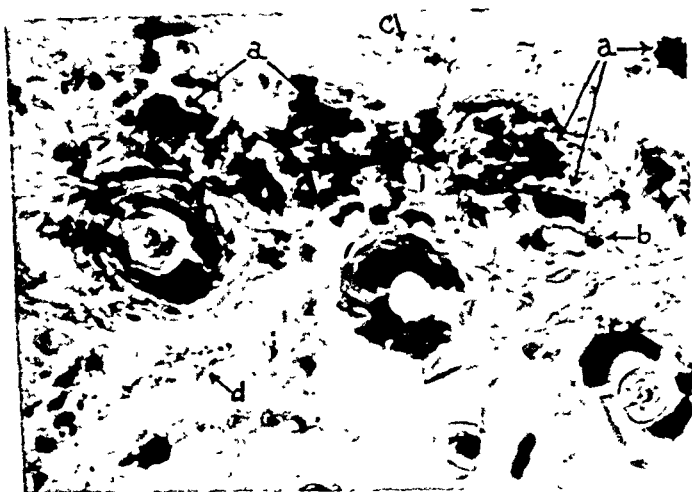
CYTOCHEMISTRY OF MAST CELLS

FIG. 1.—Section of mouse skin stained by Gomori's method for alkaline phosphatase. Mast cells (a) showing a strongly positive reaction are seen in the vicinity of hair follicles and a capillary vessel (b). In contrast, another mast cell (c) contains only a few positive granules. Dispersed extracellular granules (d) also contain the enzyme. ×400.

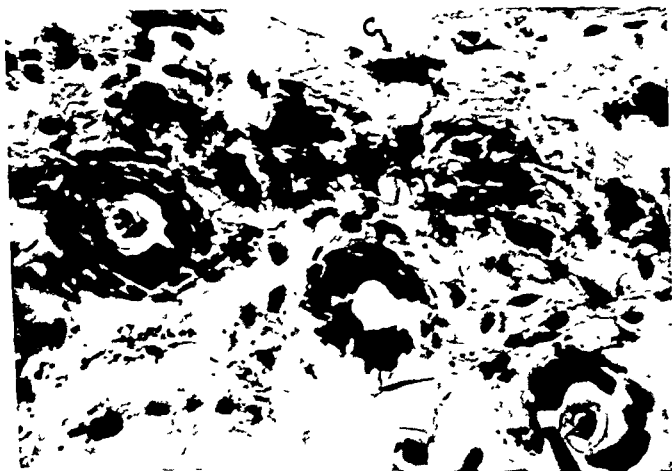FIG. 2.—The same section counterstained with toluidine blue. The mast cell (c) is now seen to be crowded with additional granules. In yellow light these appear dark crimson (metachromasia), although the monochrome photograph does not distinguish them from the dark blue nuclei or the structures already stained black by Gomori's method. ×400.

3. *Mast cells*

The opportunity was taken of confirming the results of previous workers concerning the relative number and distribution of mast cells in the loose connective tissues of the species examined. Particular attention was paid to the observation of Cramer and Simpson that an increased number of mast cells is to be found in the skin of mice which have been painted with a carcinogenic hydrocarbon. In such material the subcutaneous tissues may be packed with mast cells, but as Cramer and Simpson observe, unless a metachromatic stain is used, the mast cells may be entirely overlooked.

DISCUSSION

In recent years the enzymatic activity of mast cells has aroused considerable interest and has been the subject of a number of investigations (Noback and Montagna, 1946; Wislocki and Dempsey, 1946; Paff, Montagna and Bloom, 1947; Pritchard, 1947; Montagna and Noback, 1948). As a result of these and the present investigation, it is evident that the granules of at least some of the mast cells of mouse, rat, dog and man contain alkaline phosphatase, but it is equally clear that the presence of the enzyme is by no means a constant property of mast cells, even in these particular species. It is therefore unlikely that the mast cells in the rat contribute significantly to the intense alkaline phosphatase activity which accompanies the terminal stages of wound repair in this animal (Fell and Danielli). Moreover the general failure to find the enzyme in association with free chromotrope substance renders improbable the concept which it was desired to test in the present investigation, that the mast cells play a dual role in the regulation of cellular proliferation by secreting into the tissues one factor for growth (F.C.S.) and another for differentiation (alkaline phosphatase).

An alternative explanation may however be considered for the capricious occurrence of alkaline phosphatase in mast cells lying near capillaries. If it is true that histogenous mast cells develop principally from precursors in the neighbourhood of the smaller blood-vessels (Bäumer, 1896; Heller, 1904; Holmgren, 1946-47), then the phosphatase-containing mast cells which we observed in this situation may have been immature forms and the enzyme may be concerned with the maturation of the cell rather than with its ultimate functional activity. The suggestion is therefore made that the alkaline phosphatase in mast cells is primarily concerned with the elaboration of the characteristic metachromatic substance of the mast cell granules, a view which is in harmony with the known ability of alkaline phosphatase to participate in the synthesis of various other cytoplasmic inclusions (Bodian and Mellors, 1945; Dempsey and Wislocki, 1945; Bradfield, 1946; Jeener, 1947).

SUMMARY

1. Mast cells in tissue sections from man, mouse, rat, cat, dog, horse, rabbit, guinea-pig, mole, fowl, frog, axolotl and brown trout were examined for the presence of alkaline phosphatase by means of the Gomori technique.

2. Only in the mouse and rat was satisfactory evidence obtained for the presence of alkaline phosphatase in mast cells, and even in these two species only a proportion of mast cells contained granules in their cytoplasm which stained positively for the enzyme. These cells were found chiefly near capillaries.

3. With minor exceptions, for which the evidence was equivocal, " free chromotrope substance " (F.C.S.), which is believed to be derived from the granules of mast cells, failed to stain for alkaline phosphatase.

4. The above data do not support the view which the investigation was designed to test, that the mast cells are concerned in the regulation of reparative growth by the liberation into the tissues of factors for the promotion of growth (F.C.S.) and tissue differentiation (alkaline phosphatase).

5. If the view is correct that histogenous mast cells arise from precursors in the neighourhood of capillaries, the phosphatase-containing mast cells found in this situation in the mouse and rat may be immature cells and the enzyme contained in their granules may be concerned with the actual formation of the metachromatic material by which mast cells are generally recognised.

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616.831.9—002.4—018.9:615.778 (Streptomycin)

CHANGES IN THE MENINGEAL VESSELS IN ACUTE AND CHRONIC (STREPTOMYCIN - TREATED) TUBERCULOUS MENINGITIS

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(PLATES LX-LXIII)

INTEREST in the pathology of tuberculous meningitis has recently been stimulated by the introduction of streptomycin therapy. Apart from trying to find the causes for failure of treatment and investigating effects of the drug, the pathologist now has the chance to study chronic, healing and healed lesions in the meninges following the prolongation of life by streptomycin treatment. This paper deals with a histological study of the meningeal vessels in 20 treated and 26 untreated cases of tuberculous meningitis.

In trying to assess any changes in treated cases, it was found essential for comparison to go back to material from untreated cases. Arteritis is a prominent feature of the acute meningeal lesion and was first recorded nearly 70 years ago by Baumgarten (1881). Since then there has appeared a comparatively small literature on the subject. This was partly reviewed recently by Winkelman and Moore (1940), who added an excellent study of 5 new cases. Opinion has altered from Hektoen's (1896) view that the arteritis resulted from tuberculous infection from within the vessels. Nowadays most pathologists are in agreement with Rich and McCordock (1933) that the arteries are affected chiefly from without in their passage through the subarachnoid exudate.

I. UNTREATED CASES

The findings in 26 fatal cases of acute tuberculous meningitis occurring in Hammersmith Hospital before the introduction of streptomycin treatment are given below. Only vessels traversing exudate were found to be altered, *i.e.* the basal vessels and their proximal branches. Arteries traversing sulci of the vertex where there was no exudate were unaffected. The grosser the exudate, the more profound was the arteritis, which was thus particularly marked in sections of the interpeduncular fossa, Sylvian fissures

and midbrain. The pial veins traversing exudate showed an intense phlebitis; in fact in borderland zones between normal and infected pia-arachnoid, phlebitis preceded arteritis.

The most constant finding in affected arteries (fig. 1) was a gross swelling of the adventitial coat of vessels of all sizes except the very largest, due chiefly to local proliferation within the adventitia of reticulin-forming epithelioid cells (figs. 2-4). To a lesser extent, lymphocytes and mononuclear phagocytes of the surrounding exudate were caught up between peripheral adventitial collagen fibres. The earliest lesion detected in foci of minimal exudate was an œdema of the adventitial coat. Examples were seen of classical tuberculous change consisting of radially arranged epithelioid cells and Langhans' multinucleated giant cells (fig. 3). In general, the cytology of the adventitia corresponded with that of the exudate. Acid-fast bacilli were found only infrequently in the proliferated adventitial coat. The proliferation did not always involve the whole circumference of the vessel but varied directly in quantity as well as quality with the distribution of the surrounding exudate (fig. 2). At times it suffered a complete caseous necrosis.

Lesions of the intima varied from slight lifting of the endothelium by underlying œdema (fig. 2) to marked lifting by an infiltration with lymphocytes and large mononuclears (fig. 5), producing a beaded appearance under the very low power of the microscope. The smallest arteries were occasionally completely occluded by cellular infiltration of the intima. In 20 of the 26 cases, examples were seen of the fibrinoid change described by Askanazy (1910). Fibrin was deposited either focally (fig. 6) or as a bulky ring (fig. 7) between the intimal endothelium and the stretched internal elastic lamina. This lesion, which was associated with a meningeal exudate rich in fibrin, was seen in areas where the meningitis was most fulminating. No recognisable intimal tubercles were seen, but they have been recorded by previous writers (Winkelman and Moore). No acid-fast bacilli were seen in the intima. In 8 of the 26 cases occasional examples were found of fibrocellular intimal endarteritis (figs. 8 and 9). This consisted of a subendothelial proliferation of fibroblasts with formation of reticulin and sparse collagen but no elastic fibres. Sometimes the intimal thickening was lunate, often in relation to an adventitial caseous focus (fig. 8). In other cases it was concentric, involving the whole intimal circumference (fig. 9). It is tempting to speculate whether this type of lesion might not represent organisation of a toxic subendothelial intimal fibrinous exudate. Fig. 10 shows that though the cases presenting some degree of fibrous endarteritis mostly fall into the older age groups with longer duration of illness, there are exceptions. Thus one child only 2 years old who had been ill for 3 weeks and one child 10 years old who had been ill for 2 weeks both showed examples of fibrous endarteritis.

The media was mostly unaffected. In some cases it showed a

fibrinoid necrosis associated with intimal fibrin deposition. In occasional vessels traversing a large caseous focus the media was involved in the generalised massive necrosis affecting the other coats and the lumen. This type of lesion and advanced intimal cellular infiltration of smallest arteries were the two causes found of complete vascular obstruction. Completely occlusive simple thrombosis of arteries was not seen.
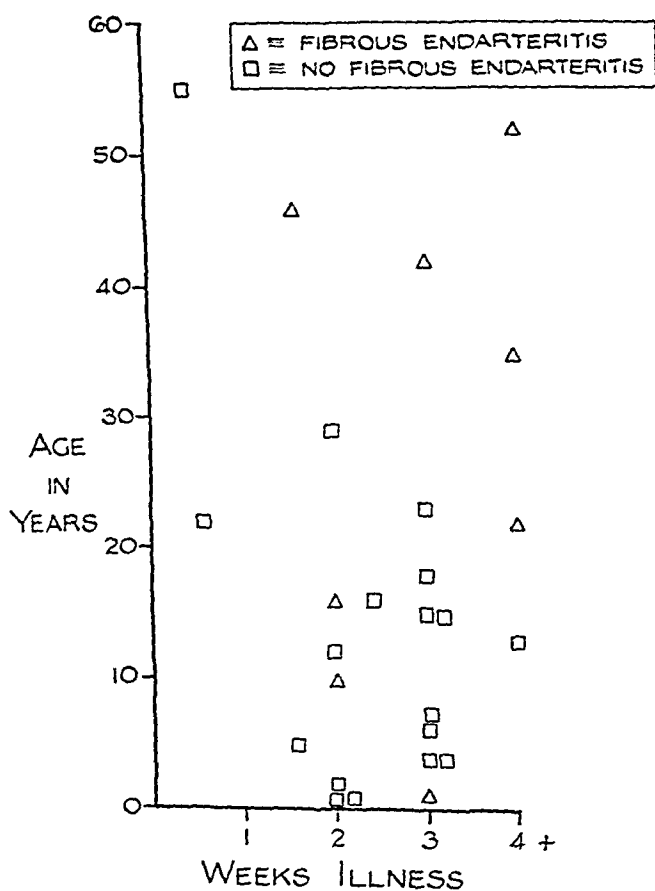

FIG. 10 —Incidence of fibrous endarteritis in 26 untreated cases in relation to age and weeks of illness.

The commonest lesion of the veins traversing tuberculous exudate was thickening of their thin walls by widespread infiltration with lymphocytes and to a lesser degree by proliferation of epithelioid cells (fig. 6). Some veins showed focal adventitial tubercles only. A few showed a " beaded " intimal cellular infiltration as seen in the arteries. Thrombosis was not seen, though occlusion by caseous necrosis occurred, as in the arteries, in veins traversing massive caseous foci.

Comment on untreated cases

The above findings confirm the generally held impression that most of the changes in the arteries and veins result from the tuberculous inflammation surrounding them. Not all the changes are specifically tuberculous. Similar intimal fibrin deposits were seen by Cairns and Russell (1946) in acute pneumococcal meningitis and the same authors described fibrocellular endarteritis in 2 cases of treated pneumococcal meningitis surviving for 7 or 8 weeks. Varying degrees of intimal fibrin deposition and medial fibrinoid necrosis can be seen in the small arteries of the meso-appendix in cases of suppurative appendicitis. Intimal fibrous endarteritis of vessels traversing the base of a chronic peptic ulcer is commonplace. Similar lesions are seen in visceral
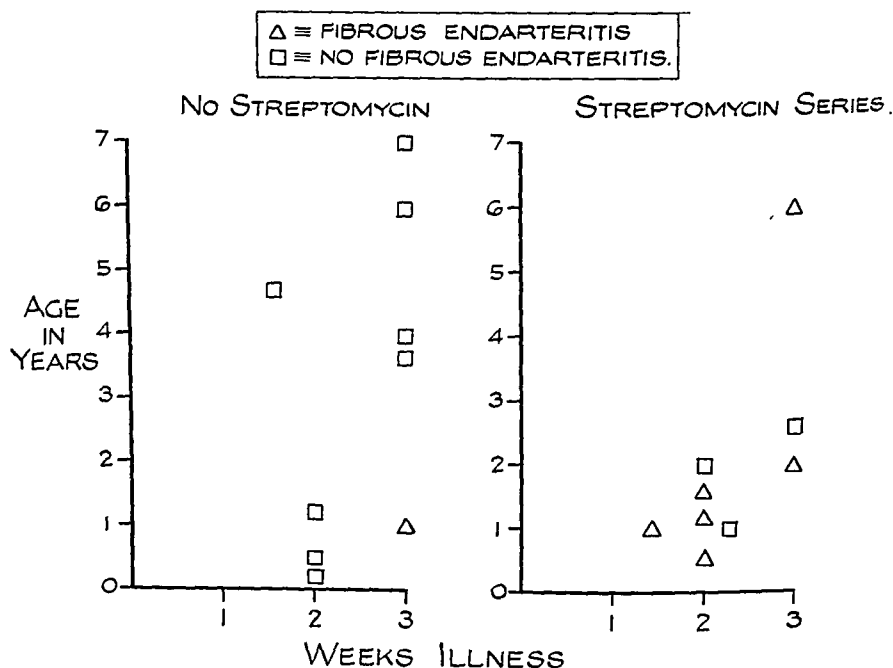
FIG. 11.—Incidence of fibrous endarteritis in 9 streptomycin-treated and 9 untreated cases under 7 years of age in relation to age and weeks of illness.

arteries in nephritis, in hypertension and in polyarteritis. It would seem, therefore, that these changes are merely a common expression of the reaction of arteries to a variety of stimuli—bacterial, toxic, hypertensive or allergic, separately or in varying combination.

II. TREATED CASES

Of 42 cases of tuberculous meningitis treated with streptomycin at Hammersmith Hospital by Dr D. MacCarthy and Dr T. P. Mann during 2 years from January 1947, post-mortems were obtained in 20 of the 25 cases who had died by December 1948. Of these 20 (all under 7 years of age) 9 had died within 4 weeks, some of whom

had received only very few doses of the drug. The rest had survived for periods ranging from 10 to 54 weeks.

The 9 cases dying within 4 weeks of the onset of symptoms showed no qualitative differences in their meningeal vessels from the untreated cases. Intimal fibrin deposits were seen less frequently—in 3 out of 9 cases as compared with 20 out of 26 untreated controls. Examples of fibrous endarteritis were seen in 6 of these 9 cases. Among the untreated controls there were, by chance, 9 cases under 7 years of
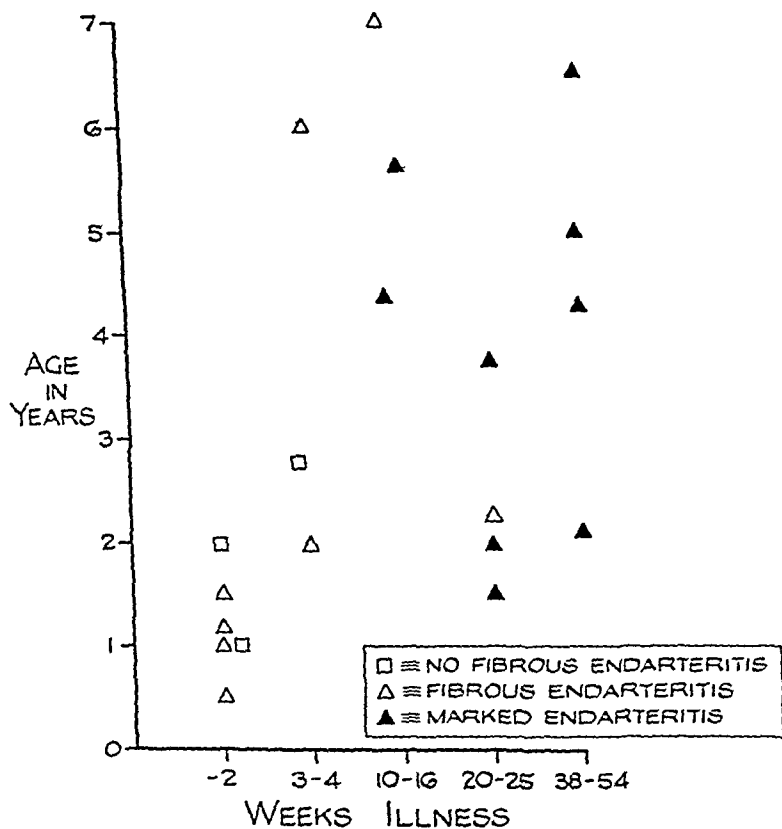
Fig. 12.—Incidence of marked and moderate endarteritis in 20 streptomycin treated cases in relation to age and weeks of illness.

age with a history of less than 4 weeks' illness : only one of these showed endarteritis. The findings in these two groups are compared in fig. 11. The meningeal vessels of all cases surviving 10 weeks or more showed striking changes (fig. 12). These only affected vessels traversing exudate, which by now was somewhat fibrous. The adventitia was replaced by a thick ring of hyalinised collagen (fig. 13), the media of most vessels appeared normal, the intima of most vessels was grossly thickened by fibrous tissue, usually lunate (figs. 13 and 14), sometimes concentric (fig. 15). There was a resulting gross reduction of the lumen of arteries of all sizes. In cases which had survived

16 weeks or more, the fibrous endarteritis differed from that seen in untreated and short-lived treated cases in the frequent additional development of new elastic fibrils (fig. 16). These often formed a new extra internal elastic lamina just under the intimal endothelium (figs. 13, 14 and 16-18), recalling the similar picture seen in syphilitic meningeal arteritis. These changes were seen in both vessels traversing completely fibrosed " healed " exudate (figs. 13 and 14) and in vessels traversing stagnant cellular fibrino-caseous exudate (fig. 15). They were also seen in a boy aged $1\frac{1}{2}$ years who was given only 1 months' streptomycin treatment but who survived a further 5 months without any treatment. A girl aged $6\frac{1}{2}$ years who survived 1 year showed atheromatous collections of lipoid-laden phagocytes deep in the markedly thickened fibrous intima of one vertebral artery lying over the medulla (fig. 19). Patients dying of a relapse—a fresh attack of meningitis—showed a double lesion. Scarred vessels as described above were unchanged (fig. 17), presumably because their hyalinised adventitia was unable to respond by cellular proliferation and at the same time formed a protective barrier to the intima. Unscarred vessels traversing fresh exudate showed an adventitial arteritis indistinguishable from that seen in untreated cases. Intimal fibrin deposits were not seen.

Though occasional examples were found of fibrosed veins (fig. 18) in the long-surviving cases, the majority showed either normal or actively inflamed veins. In relapse, active phlebitis was prominent.

Comment on treated cases

No evidence was found to suggest that streptomycin had any direct effect on the vessels. The findings are all explicable by the prolongation of life. The adventitial reticulin-forming epithelioid-cell proliferation is eventually replaced by hyalinised collagen, the intimal fibrin by fibrous tissue, and from 16 weeks onwards elastic fibrils develop in the fibrous intima. These changes were seen to a certain extent in untreated cases and would almost certainly be found in the rare cases of spontaneously long-surviving chronic tuberculous meningitis. Examination of a few slides of cerebral and cerebellar tuberculomas showed endarteritis but no elastosis of local vessels. As with the acute lesion, the intimal changes are by no means specific and are seen in a variety of conditions affecting the vessels of the brain and viscera, including syphilitic arteritis, chronic non-specific inflammations, senile involution, hypertension and, as recently demonstrated by Harrison (1948), organisation of intra-arterial thrombi. Both in acute and chronic tuberculous meningitis, the occlusive intimal changes may give rise to widespread ischæmic lesions of the brain and spinal cord (Smith and Daniel, 1947; Smith, Vollum and Cairns, 1948).

SUMMARY

The histological changes in the meningeal vessels of 20 cases of tuberculous meningitis treated with streptomycin were compared with those in 26 untreated cases. The prolongation of life as a result of streptomycin therapy resulted in an increase of fibrous endarteritis. There was additional intimal elastic-fibre formation in cases surviving more than four months.

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MENINGEAL VESSELS IN TUBERCULOUS MENINGITIS

FIG. 1.—Female aged 1¼ years; 14 days illness. Inflammatory swelling of adventitia of pial arteries traversing fibrino-caseous exudate in pia-arachnoid over base of brain. Hæmalum and eosin. ×28.

FIG. 2.—Female aged 15 years; 3 weeks illness. Mononuclear-cell proliferation involving three-quarters of the circumference of the adventitial coat of an artery traversing focally caseating exudate overlying cerebellum; early focal œdema and lymphocyte infiltration of intima. H. and E. ×112.
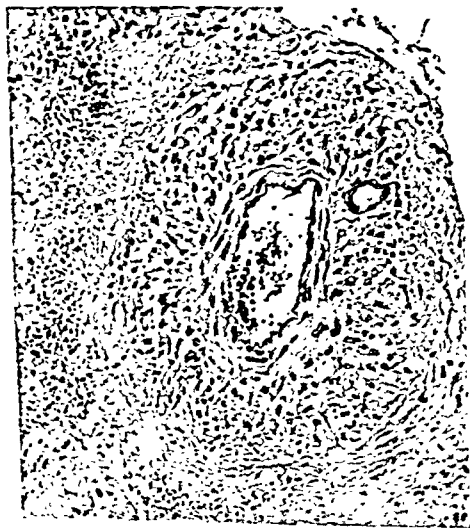
FIG. 3.—Male aged 4 months; 2 weeks illness. Concentric epithelioid-cell proliferation, with giant-cell formation, in the adventitia of an artery traversing caseating exudate over base of brain. H. and E. ×130.

FIG. 4.—Reticulin-fibre formation in adventitial coat of arteries including half of the one illustrated in fig. 3. Gordon and Sweet's stain. ×130.

MENINGEAL VESSELS IN TUBERCULOUS MENINGITIS

FIG. 5.—Female aged 29 years; 2 weeks illness. Verrucose intimal swelling, by subendothelial lymphocytic and fibrin-droplet infiltration, of an artery traversing caseating cellular exudate over cerebellum. H. and E. ×130.
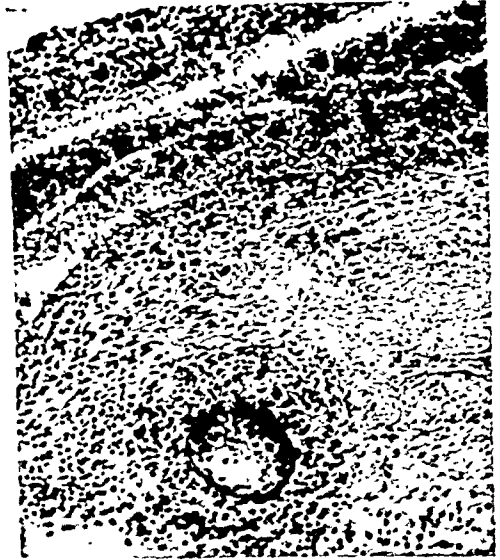
FIG. 6.—Female aged 15 years; 3 weeks illness. Lymphocytic infiltration and mono-nuclear-cell proliferation in wall of vein (above) and arteriole (below) traversing fibrino-caseous exudate in Sylvian fissure; focal intimal fibrinoid change in intima and media of the arteriole. H. and E. ×110.
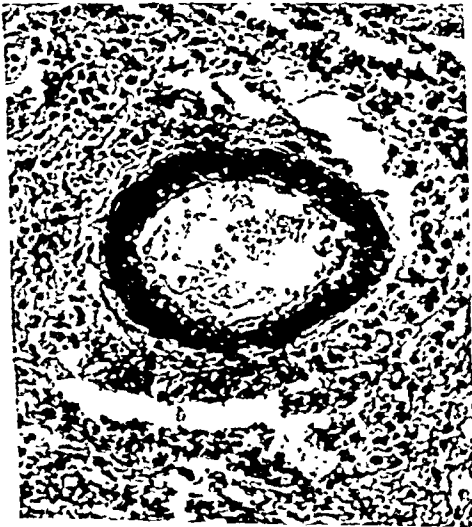
FIG. 7.—Female aged 16 years; 2½ weeks illness. Concentric subendothelial intimal fibrin deposition, outside which runs the stretched fine unstained internal elastic lamina of an arteriole traversing fibrino-cellular exudate in the Sylvian fissure. Mallory's phosphotungstic-acid hæmatoxylin. ×220.

FIG. 8.—Female aged 16 years; 2 weeks illness. Focal subendothelial intimal fibrocellular proliferation, lunate in shape, lying beneath focal adventitial caseation of an artery traversing the Sylvian fissure. H. and E. ×60.

MENINGEAL VESSELS IN TUBERCULOUS MENINGITIS

FIG. 9.—Male aged 2 years; 2 weeks illness. Concentric obliterative intimal subendothelial fibrocellular hyperplasia (endarteritis fibrosa) of an artery traversing fibrino-caseous exudate over midbrain; œdema and cellular infiltration of intima of neighbouring vessels. H. and E. ×60.
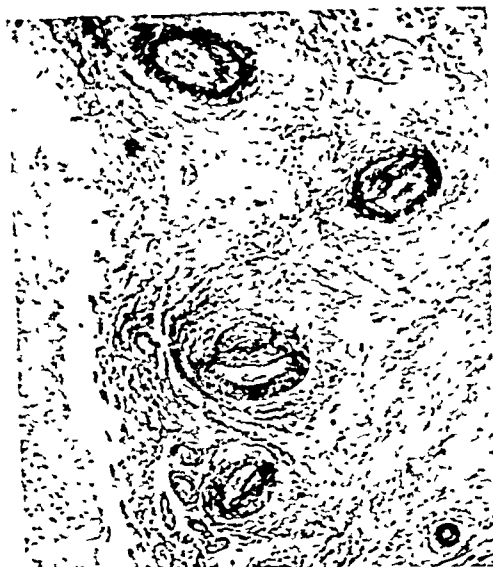

FIG. 13.—Male aged 2 years; 6 months illness (streptomycin-treated). Hyalinised collagenous exudate in interpeduncular fossa traversed by arteries showing a thickened hyalinised adventitia and lunate obliterating fibro-elastic intimal endarteritis. Verhoef and van Gieson. ×48.
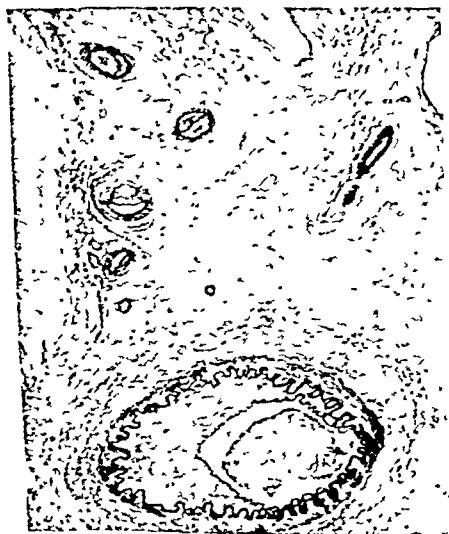
FIG. 14.—Same case as in fig. 13, showing similar changes in varying sized arteries and similar formation of an additional internal elastic lamina. Verhoef and van Gieson. ×48.
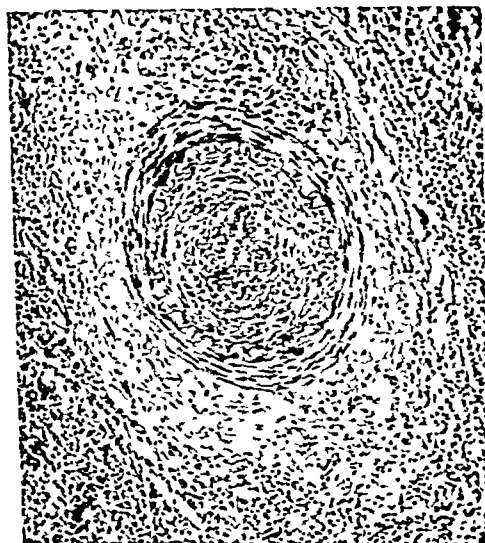
FIG. 15.—Male aged 4½ years; 4 months illness (streptomycin-treated). Fibrous, poorly cellular thickening of adventitia, healthy media and obliterative fibrosis of intima of an artery traversing stagnant caseating cellular exudate over base of brain. H. and E. ×95.
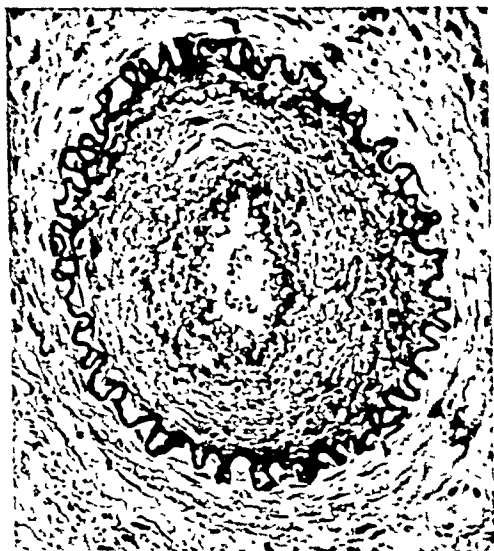
MENINGEAL VESSELS IN TUBERCULOUS MENINGITIS

FIG. 16.—Same vessel as in fig. 15, showing newly formed intimal elastic fibrils. Weigert's elastic stain and neutral red. × 200.

FIG. 17.—Female aged 6¼ years; 1 years illness (streptomycin-treated); relapsed meningitis. Fibrocellular exudate in interpeduncular fossa traversed by arteries showing fibro-elastic and fibrous endarteritis, broken elastic laminæ and a scarred acellular adventitia. Weigert's elastic stain and neutral red. × 22.

FIG. 18.—Male aged 4 years; 9 months illness (streptomycin-treated). Fibrosed exudate in interpeduncular fossa, traversed below and to left by an artery showing fibrous endarteritis and collagenous thickening of the adventitia, above and to right by a vein whose wall is grossly thickened by fibrous scar tissue. Verhoef and van Gieson. × 48.

FIG. 19.—Female aged 6¼ years; 1 years illness (streptomycin-treated); relapsed meningitis. Large lipoid-laden foamy mononuclear phagocytes in the depths of the thickened intima—atheroma of one vertebral artery running over medulla. Verhoef and van Gieson. × 130.

SHORT ARTICLES

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A CASE OF THE ARNOLD-CHIARI MALFORMATION OF THE HIND BRAIN

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(PLATES LXIV and LXV)

The Arnold-Chiari malformation is a congenital anomaly in which a tongue of cerebello-medullary tissue is displaced into the cervical vertebral canal where it lies dorsal to the spinal cord. Only about 180 cases are reported in the literature, where it has come into prominence as a result of the therapeutic efforts of the neurosurgeon. The degree of deformity is very variable but the following case shows the typical features of the more severe type of lesion associated with spina bifida and meningo-myelocele and resulting congenital hydrocephalus.

In these cases routine autopsy methods in which the brain and cord are removed separately are unsatisfactory because of the inevitable resulting damage to the deformed medullary region. It is advisable to conduct the examination of the central nervous system and its supporting structures as follows. Partial fixation of the brain *in situ* is first obtained by perfusion of both carotids with formalin. The skull is then opened and after division of the cerebral peduncles both hemispheres are removed together. Finally the intact tentorium cerebelli with the whole of the posterior cranial fossa and the whole of the vertebral column with all their contents are removed in one piece and their fixation completed in formalin. Dissection, radiography and histology of the specimen can then be undertaken.

CASE REPORT

Clinical history

C. J. C., a female infant, was born at term on 23rd June 1948. The mother, a primi-gravida aged 24, had hyperemesis but no other illness during pregnancy. Labour was normal except that episiotomy was done because of the large size of the head. The infant weighed 2800 g. There was extreme moulding with no caput but with widening of the sutures on the upper aspect of the skull. The head measured 34 cm. in circumference. There was a large intact bulging meningo-myelocele in the dorso-lumbar region overlying a spina bifida. This sloughed in its central part within a few days of birth. Paralysis of the lower limbs and marked sacral and lumbar œdema were noted within two weeks. As the hydrocephalus increased the general condition of the infant gradually deteriorated. During the last three weeks of life there was pyrexia to the extent of 100·8° F. The infant died on 19th August 1948, aged eight weeks.

Post-mortem examination (no. 4457)

The body, examined 19 hours after death, was that of a young female infant 53 cm. in length and weighing 3750 g. There was a severe degree of hydrocephalus and the head measured 45 cm. in circumference. There was a large

dorso-lumbar spina bifida with a collapsed meningo-myelocele which was rhomboid in shape and measured 5 cm. in length and 3 cm. in width. It appeared completely epithelialised except for an area near the centre 0·5 cm. in diameter which was covered with a dry crust. Removal of the crust revealed a fistulous opening into the meningo-myelocele. Gross and microscopical studies of the cardiovascular, respiratory, alimentary, genito-urinary, endocrine and reticulo-endothelial systems showed no significant abnormalities.

The vault of the *skull* showed severe cranio-lacunia, with numerous paper-thin areas separated by bony ridges. The suture lines were separated and both fontanelles were widely open. The base of the skull showed no abnormality but the tentorium cerebelli seen from above was depressed and concave.

The *vertebral column*, 22 cm. long, showed an obvious lordosis and marked right convex scoliosis in the mid-dorsal region. There was also severe dorso-lumbar kyphosis corresponding to the spina bifida. The cervical vertebræ were normal. Thoracic vertebræ 6-8 showed partial fusion of their bodies, with a wedge deformity at the bulge of the mid-dorsal scoliosis. Radiologically, 11 ribs were seen on the right side but of the ten present on the left, nos. 6 and 7 were fused at their origins and arose from the 6th, 7th and 8th vertebræ. The twelve thoracic and five lumbar vertebræ could be identified but the 10th, 11th, 12th thoracic and all the lumbar vertebræ had ununited laminæ and the result was a dorso-lumbar spina bifida. The five sacral and the coccygeal vertebræ were normal. Within the vertebral canal in the region of the scoliosis just above the spina bifida the dura was very thick and firmly bound to the fused vertebral bodies. Fibrous prolongations filled the pits of the bone surface, which appeared rough when the membranes were torn away. In the caudal part of the meningo-myelocele the greater part of the spinal cord was seen to be displaced dorsally and to the left. The reason was found in the presence of a rod of bone 2 mm. in diameter which ran obliquely caudal and to the right from the body of the 4th lumbar vertebra in the mid-line dorsally across the vertebral canal to join the lamina of the first sacral vertebra. As it traversed the vertebral canal it was invested by a meningeal sleeve.

The *cerebral hemispheres* showed extreme hydrocephalus (fig. 1). A mid-coronal section after fixation measured 11·5 cm. from side to side and 10 cm. from above to the inferior aspect of the temporal lobes. The lateral ventricles were extremely dilated, measuring up to 5 cm. in transverse diameter. The foramina of Monro were approximately 2 cm. in diameter. The third ventricle, lying above the tentorial notch, was dilated, but the aqueduct of Sylvius in and below it was not. There was no obstruction within the iter. The cerebral convolutions were so flattened that it was difficult to observe if there was any microgyria. One or two small areas of hæmorrhage measuring up to 0·3 cm. in diameter were obvious in the leptomeninges of the hemispheres but no macroscopical exudate was seen here.

Cerebellum, pons and *medulla* showed the characteristic Arnold-Chiari malformation. These structures were deformed and had been forced caudally from their normal position. The cerebellum itself failed to show well-developed anatomical features, although vermis and hemispheres were crudely indicated. From the tonsillar region a flattened tongue of cerebellar tissue measuring 1·7 cm. wide and 6 cm. long extended through the foramen magnum into the dorsal part of the cervical spinal canal to the level of the 7th cervical vertebra (fig. 2). This tongue of tissue showed at its cephalic end a dorsal semi-circumferential ridge where the foramen magnum was larger than the cervical portion of the vertebral canal. Ventral to this tongue, a smaller tongue of medullary tissue projected downwards for 5 cm. and between the two lay the elongated and broadened fourth ventricle. The caudal end of the medullary tongue showed a tiny opening which was the beginning of the central canal of the spinal cord. On each side of the cerebellar tongue the choroid plexus of the

ARNOLD-CHIARI MALFORMATION

FIG. 1.—Coronal sections through the cerebral hemispheres, showing the extreme degree of hydrocephalus. ×0·6.

fourth ventricle extended dorsally in two lateral portions which met in the mid-line just cephalic to the tip of the tongue. The floor of the fourth ventricle showed a prominent median groove but the typical features of the caudal half of the rhomboid fossa could not be identified with certainty. The cephalic part of the floor of the fourth ventricle was distorted by the cerebellar tongue. The ventral aspect of the medulla similarly was lacking in well-marked anatomical features. The pons was elongated, measuring 1·8 cm. from cephalic to caudal margins. The anterior ventral surface was relatively flattened. The corpora quadrigemina were represented by a simple conical tectal mass.

The *cranial nerves* were greatly elonged (fig. 3) and ran cephalad from their origin to their foramina in the skull. These distances measured 2·1 cm. for the fifth nerve, 3·1 cm. for the sixth nerve, 2·2 cm. for the facial nerve and 1·8 cm. for the auditory nerve.

At the upper end of the medullary tongue, on its ventral aspect, the *cervical cord* could be identified as an individual structure, 1·3 cm. broad at this point but only 0·1 cm. in thickness where it was compressed dorso-ventrally by pressure of the cerebello-medullary tongue. The tongue and the cervical cord together completely filled the upper cervical vertebral canal. Caudal to the cerebello-medullary tongue the lower cervical cord itself was rather enlarged, measuring 1·1 cm. in width and 0·6 cm. in thickness. The spinal cord in the mid- and lower dorsal region was very small and thin, measuring only 0·4 cm. in diameter. The cord became wider and thicker in the meningo-myelocele and, more caudally, showed an apparent diplomyelia where the greater part of its substance was diverted to the left round the abnormal bony arch. The lower end of the cord reached the level of the fifth lumbar vertebra. Beyond this, the filum terminale measured only 3 cm. in length. There was no gross hydromyelia.

The degree of cephalic direction of the *spinal nerve roots* was maximal in the upper cervical region, where it corresponded to that of the cranial nerves. As the lower dorsal region was approached this decreased and at the upper end of the meningo-myelocele the nerve roots appeared to run transversely. Further down the cord the nerve roots ran increasingly caudal, till their direction was normal in the much shortened cauda equina.

The *lepto-meninges* were thickened in the region of the mid-brain. The tongue of brain tissue within the cervical spinal canal was closely invested in quite thick highly vascular and hæmorrhagic gelatinous leptomeninges which were adherent to the dura. Although slightly less vascular as the meningo-myelocele was approached, the adhesions and gelatinous exudate remained obvious. The meningeal wall of the meningo-myelocele was intimately connected with the nerves within it. A small amount of purulent material was seen in the region of the fistulous opening.

Histology

The investing leptomeninges of the hemispheres exhibit little change. In relation to the small areas of hæmorrhage, however, there is an extensive inflammatory-cell reaction made up of large mononuclear phagocytes in which considerable amounts of iron-containing pigment are present. Extra-cellular deposits of non-iron-reacting pigment are also apparent in the brain substance just below the surface. The meninges are highly cellular in these areas and many large mononuclear phagocytes are present which, in appropriately stained frozen sections, are found to contain fat droplets. The brain substance itself is much thinned, the thinning being nearly all in the white matter. The layers of nerve cells in the cortex show little change but the fibres beneath are thinned. The ependyma, where visible, appears normal, although it is not obvious in considerable portions of some of the sections.

Sections from various portions of the meningo-myelocele show several interesting features. Epidermisation has occurred, the stratified squamous epithelium

covering all except the fistulous opening. Beneath this epithelium there is a thin layer of fibrous tissue representing the meninges, which do not show differentiation into pia-arachnoid and dura. No skin adnexa are seen here but hair follicles and hairs are present to the side of the lesion. The deeper part of the undifferentiated meninx shows a number of nerves running through it. Here the meninges are more cellular and contain numerous inflammatory cells, chiefly mononuclear, but many polymorphonuclears are also present, indicating an acute if low-grade meningitis. There is a high degree of vascularity and areas of hæmorrhage and broken-down blood pigment are obvious. In one area the meningeal inflammatory process exhibits a well-marked foreign-body reaction. A number of large multinucleated giant cells are seen, in many of which are doubly refracting bodies. A fragment of ossifying vertebral arch invested by a thin sheet of densely collagenous dura is present in the middle of this network of meninges and nerves. The dura investing the osseous portions of the vertebræ is thick and dense. The spinal cord shows considerable distortion of its normal architecture, while the central canal appears double and is some-what dilated.

DISCUSSION

Since Arnold in 1894 and Chiari in 1895 (quoted by Schwalbe and Gredig, 1906) described the first cases, a variety of views have been put foward to explain the features of the Arnold-Chiari malformation. Penfield and Coburn (1938) believed that the malformation arose during growth as the result of caudal traction on the cerebellum, brain stem and nerve roots by adhesions at the site of the spina bifida. This somewhat too ingenious explanation was supported by others (Ogryzlo, 1942 ; Ingraham and Scott, 1943), but recently Russell (1949) has presented evidence to show that this view is unacceptable. In the case here reported it is also difficult to visualise how traction on the cord and its meninges from below could produce the large tongue-like structure which lies dorsal to the lower medulla and upper part of the cervical cord and is made up of both a portion of cerebellar tissue and a fold of the upper part of the medulla. Furthermore, the incomplete development of the pons, medulla and cerebellum which this case also shows is suggestive rather of some aberration of growth. Undue support of the traction theory has perhaps resulted from the failure of other writers (Adams et al., 1941 ; Steele, 1946-47) to stress these maldevelopments, although McConnell and Parker (1938) noted marked asymmetry of the deformed cerebellum.

The mechanism of production of the accompanying hydrocephalus has also been discussed recently by Russell (op. cit.), who reaffirms her previous belief (Russell and Donald, 1935) that the hydrocephalus is secondary to the malformation itself and indeed is almost invariably present. The hydrocephalus results from the caudal displacement of the fourth ventricle foramina whereby the cerebrospinal fluid passes directly into the spinal subarachnoid space, whence it is prevented from reaching the cranial subarachnoid space because of the plugging of the foramen magnum by the tongue of cerebellum and medulla lying tightly jammed in the upper cervical spinal canal. Most of the absorption of cerebrospinal fluid by the arachnoid villi is prevented thereby and hydro-cephalus develops. In this case it should be noted that there was no dilatation of the ventricular system below the level of the tentorium cerebelli, although obstruction to the flow of cerebrospinal fluid had occurred mainly in the sub-arachnoid space at the foramen magnum. There may possibly have been some functional obstruction within the anatomically patent aqueduct, or obstruction may have resulted from the midbrain being tightly compressed into the tentorial notch.

The marked craniolacuna (Lückenschädel) present in the skull in this case is sometimes considered to be the result of pressure of the cerebral convolutions

ARNOLD-CHIARI MALFORMATION

Depressed tentorium

Groove at foramen magnum

Groove at arch of atlas

Cerebellar tongue

Cavity of opened fourth ventricle
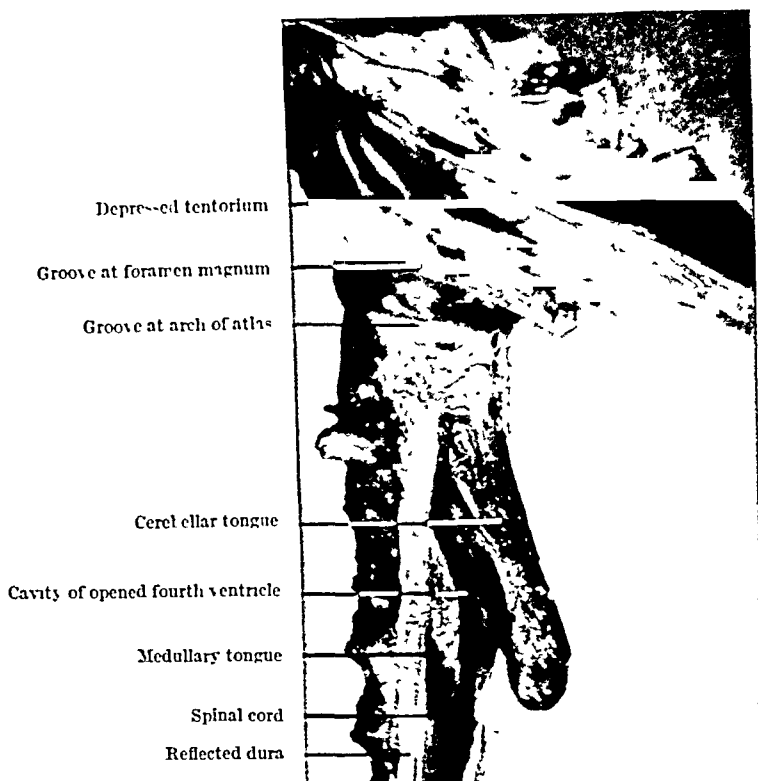
Medullary tongue

Spinal cord

Reflected dura

FIG. 2.—Brain stem and cord after removal from the base of the skull and vertebral column. In the photograph the cerebello-medullary tongue has been displaced dorsally in order to demonstrate the cavity of the fourth ventricle. × 1·5.
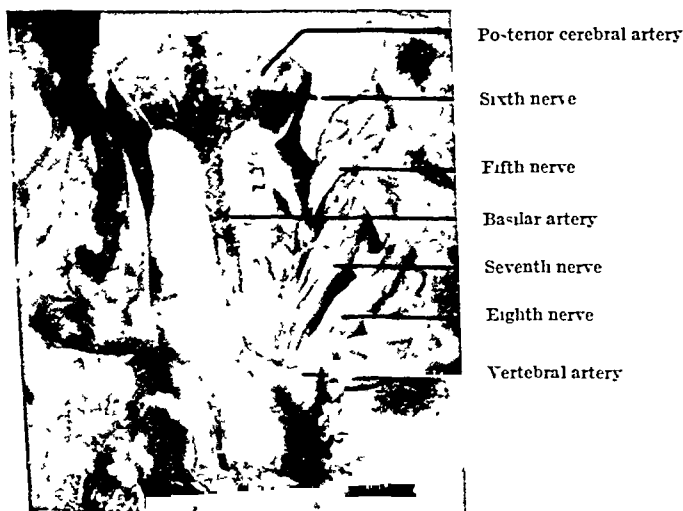

Posterior cerebral artery

Sixth nerve

Fifth nerve

Basilar artery

Seventh nerve

Eighth nerve

Vertebral artery

FIG. 3.—Ventral dissection of the brain caudal to the cut cerebral peduncles showing the elongated and distorted pons lying between the posterior cerebral and vertebral arteries. A portion of the latter on the left side is hidden by the auditory nerve, which, like the other cranial nerves, runs obliquely in a cephalic direction due to the caudal displacement of the brain stem. × 2.

due to the hydrocephalus, but Wyatt and Goldenberg (1948) believe this is unlikely, as the areas of thinning have no relation to either the convolutions or the centres of ossification. They consider that craniolacuna is a developmental defect of ossification in membrane and is not always associated with hydrocephalus.

It may be that the origin of the Arnold-Chiari malformation lies in the partial failure of development of some of the ventral parts of the hind- and midbrain. As Schwalbe and Gredig (1906) believed, it undoubtedly commences early in the developing embryo and moreover the frequent association with various other developmental anomalies is evidence pointing to an initial developmental defect intimately concerned in the formation and growth of the nervous system and its supporting structures.

SUMMARY

A case is described of the Arnold-Chiari malformation in an infant showing meningomyelocele, spina bifida with other bony abnormalities and severe hydrocephalus. The mechanism of production of the latter is discussed. Emphasis is placed on the presence of both cerebellar and medullary components in the tongue of brain tissue in the cervical vertebral canal as evidence in support of the view that the malformation is not due to caudal traction by meningeal adhesions. Rather it is assumed that there is a primary developmental defect affecting the ventral aspects of the hindbrain.

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ADRENAL CORTICAL CARCINOMA WITH METASTASES IN AN OVARIECTOMISED STRONG A MOUSE

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(PLATES LXVI and LXVII)

Spontaneous adrenal cortical carcinomata are very rare in mice. Slye *et al.* (1921) reported only 4 tumours of adrenal origin in 33,000 mice. A spontaneous adrenal cortical tumour approximately 4 mm. in diameter has also been reported in a strain C female mouse aged 24 months by Dalton *et al.* (1943-44). Although the primary tumour did not give rise to metastases, it was successfully transplanted in the same strain of mice. Recently, spontaneous malignant cortical tumours have been found in 2 males of the Minnesota line of the NH strain at 16 and 24 months respectively by Kirschbaum *et al.* (1946). These tumours were about one-third the size of the kidney and did not produce metastases. Females of the same strain a year or more old developed adrenal adenomata, the glands attaining 2-4 times the normal size.

Tumours of the adrenal cortex in ovariectomised mice of the NH strain have been observed by Gardner (1941). Of 15 mice ovariectomised at 43-65 days, 13 showed tumours of the adrenals without metastases between approximately 600 and 700 days of age. These tumours were mainly of microscopic size, but in some mice they were almost two-thirds the size of the kidney. That œstrogen was present in the ovariectomised mice was shown by the condition of the uterus and mammary glands.

Woolley *et al.* (1943) observed a high percentage of adrenal cortical tumours in ovariectomised or castrated mice of the JAX ce strain. In a series of papers Woolley and Little (1945*a-d*, 1946) gave a detailed account of the changes preceding and accompanying the formation of adrenal cortical carcinomata. Development of the accessory sex organs in females and males following the appearance of adrenal tumours showed that œstrogenic and androgenic hormones may be produced in the adrenals or in the developing tumours.

A single macroscopic lung metastasis was observed in a tumour-bearing mouse at 12 months by Woolley and Little (1945*a*), and Fekete and Little (1945) found only 8 microscopic metastases in the lungs of 43 ovariectomised ce mice between 12 and 22 months of age. No grossly visible metastases were found in other mice bearing adrenal cortical tumours in these series.

The ætiology of adrenal cortical tumours is no doubt related to an endocrine imbalance. It seems possible that a pituitary hormone of adrenocorticotropic type may operate following the removal of the gonads, causing at first hyperplasia, and finally adrenal cortical tumours. There is, however, no experimental proof for such an action of the pituitary hormone, as no increase of adrenocorticotropic hormone has so far been found in animals deprived of their gonads. It seemed, therefore, of considerable interest to inject an adrenocorticotropic
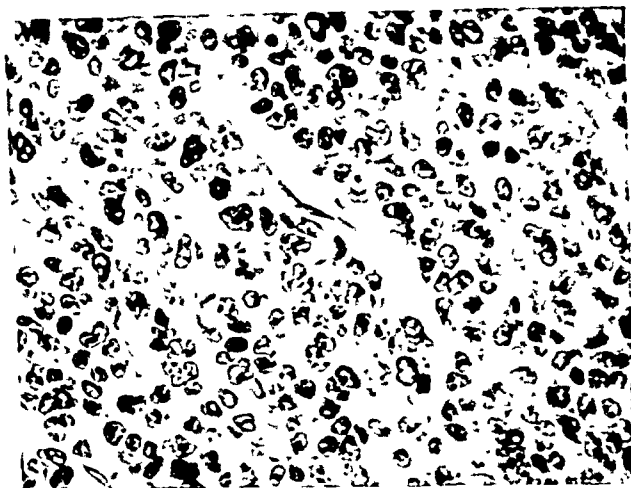
ADRENAL CARCINOMA IN A MOUSE

FIG. 1.—Liver metastasis showing the uniform nature of the tumour cells which are arranged in rows and cords and show numerous mitoses. In centre, fine connective tissue septum. Hæmatoxylin and eosin. ×540.

FIG. 2.—Left adrenal showing (lower left margin) degenerated cells of the zona fasciculata surrounding necrotic tumour tissue. Below is better preserved tumour. H. and E. ×50.
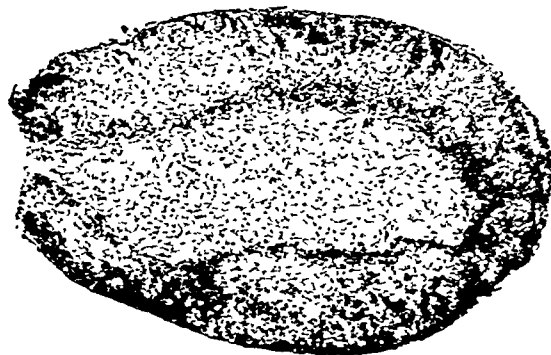

FIG. 3.—Right adrenal showing invasion of the cortex from without inwards by "type A" cells. H. and E. ×50.

pituitary extract into normal and spayed mice of various strains in order to ascertain if this hormone exhibits any influence on the formation of adrenal cortical tumours.

Amongst a group of spayed mice of different strains which received what was thought to be adequate doses of adrenocorticotropic hormone * and developed adrenal cortical tumours, one Strong A female showed a highly malignant metastasising tumour at 537 days. This mouse was ovariectomised at 3 days, weaned at 3 weeks and treated over a period of 8½ months.

At autopsy, a tumour approximately 20×12×12 mm. was found in the left flank. The left kidney, which was embedded in the growth, contained numerous metastatic nodules and was displaced downwards. It could be easily enucleated from the main mass of the tumour, which covered its ventral surface like a sheet. Anterior to this a pyramid-like nodule 6×3×2 mm. was attached on a broad base to the tumour. This nodule was identified histologically as the left adrenal, from which the growth had evidently arisen.

The right kidney also contained numerous metastatic nodules. The right adrenal was found approximately in its normal position. It was somewhat enlarged, but not invaded macroscopically.

The much enlarged liver contained numerous metastases. In addition, many small nodules were found in the pelvis, some of them adhering to the thread-like uterine horns. The growth had also extended into the left pleural cavity through the diaphragm, forming a nodule measuring 4×3×1·5 mm. in the upper part of the left pleural cavity. There were no metastases in the lungs or elsewhere and no general involvement of the lymphatic system. The spleen and thymus were tumour-free.

Histology

The structure of the tumour is seen to best advantage in the liver metastases fixed in Susa.

1. The liver contains numerous nodules disseminated throughout its parenchyma. The tumour cells (fig. 1) are mainly polyhedral and often without distinct cell borders. A follicle-like arrangement can be seen, but the cells are usually arranged in rows or cords. The protoplasm is slightly basophilic and the nuclei show variability in size and shape. Each contains one distinct or several less distinct nucleoli. Mitoses are abundant. Laidlaw's stain reveals a fine reticulum encircling groups of cells or even single cells.

2. The left adrenal (fig. 2) is recognised by the presence of a few degenerate foamy cells of the zona glomerulosa and zona fasciculata lying beneath its capsule and forming a rim to a nodule composed of almost completely degenerated tumour cells and containing a few scattered calcified foci. Deeper areas show cells with fairly well preserved nuclei and typical arrangement. At the base the nodule is connected with the main tumour surrounding the left kidney.

3. The right adrenal (figs. 3 and 4) is free from malignant growth. There is, however, an extensive replacement of the cortex by the " type A " cells of Woolley and Little (1945a), which are regarded by them as non-neoplastic. These irregular cells, with round or fusiform nuclei and scanty protoplasm, are found scattered over most of the cortical area. In some regions they were subcapsular in position, with finger-like processes spreading from the periphery into the zona glomerulosa (fig. 3). At one pole of the adrenal they occupy the whole area of the cortex as far as the medulla, ending abruptly in the boundary zone.

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4. Tumour tissue which had invaded the kidneys and the outer muscle layer of the stomach shows the same histological structure as the liver secondaries.

DISCUSSION

Some difficulty was experienced in tracing the origin of this tumour, which was so widely disseminated throughout the abdomen. In strong support of an adrenal cortical origin was the fact that the left adrenal was almost completely changed into a tumour nodule showing advanced necrosis. This suggested that it was most probably the first organ to be involved in the malignant process. It would seem that the initial cortical tumour broke through the capsule at the caudal pole of the adrenal, giving rise to the main mass of growth outside the adrenal and remaining attached to it at the time of death. The right adrenal, although not neoplastic, was heavily invaded by Woolley and Little's "type A" cells, indicating that a generally favourable state for the development of adrenal tumours existed within the body.

The structure and arrangement of the left adrenal tumour leave no doubt as to its cortical origin. When compared with a localised adrenal cortical tumour (4 × 2 mm.) found in the right adrenal of a gonadectomised and hormone-treated stock female mouse at the age of 514 days (figs. 5 and 6), the present tumour differs in that it is more anaplastic, shows more numerous mitotic figures, and has less well developed connective tissue septa.

The time of appearance of the tumour is well within the range of appearance of cortical tumours in gonadectomised mice of the JAX ce strain. It is, therefore, not possible to conclude that the injected hormone accelerated the development of this or other tumours which arose in our gonadectomised mice. But what seems to be unusual is the expansion and spreading capacity of the adrenal carcinoma here described. It is also interesting to note that malignant tumours may develop after gonadectomy, not only in the JAX ce and NH strains but also in Strong A and CBA strains and in mice of mixed stock, at advanced ages.

There was no evidence of an œstrogenic stimulation as judged by the condition of the uterus, but it was rather surprising that one week before death a pure œstrous smear occurred, and became di-œstrous on the following day.

SUMMARY

A case of a highly malignant metastasising carcinoma of the adrenal cortex in a gonadectomised Strong A female mouse treated with adrenocorticotropic hormone is described. Adrenal cortical tumours can easily be induced by gonadectomy in the JAX ce and NH strains of mice, but, so far as we are aware, no cortical tumour has been described in the Strong A strain, and in no strain one of such malignancy as to involve the liver, kidneys, outer muscle layer of the stomach and pleural cavity.

The appearance of such a tumour in a spayed mouse treated with adreno-corticotropic hormone may however be coincidental.

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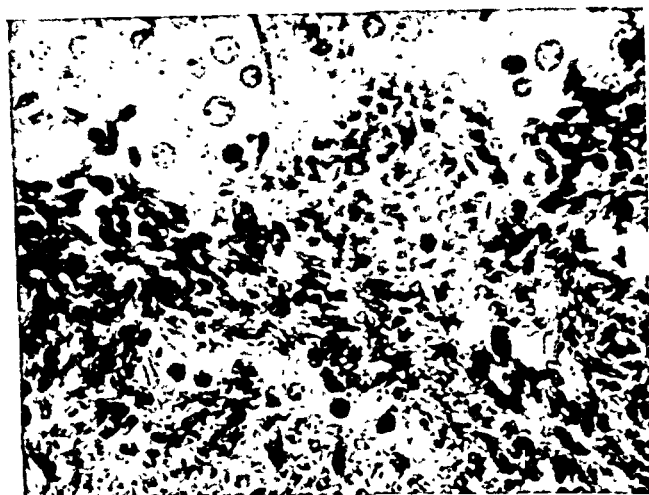
ADRENAL CARCINOMA IN A MOUSE

FIG. 4.—Same as fig. 3. High-power view of the boundary between the cortex and medulla showing "type A" cells (below) invading the cortex. H. and E. ×470.

FIG. 5.—Both adrenals of an ovariectomised stock mouse at 514 days of age. Right adrenal (right) shows a localised adrenal cortical tumour. Left adrenal (left) shows less advanced changes. H. and E. ×9.
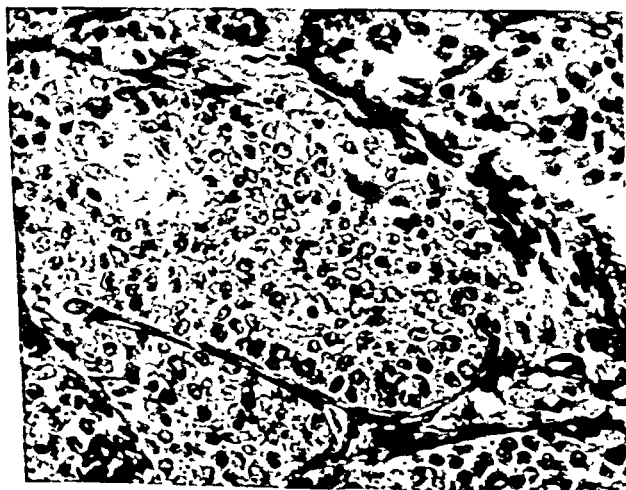

FIG. 6.—Section of tumour of right adrenal shown in fig. 5. Tumour cells arranged in cords surrounded by connective tissue. Large, vesicular "type B" cells (top right) are intermingled with the main mass of tumour cells. H. and E. ×470.

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$$578 . 65 : 576 . 852 . 211$$

THE STAINING OF TUBERCLE BACILLI WITH SUDAN BLACK B

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(PLATE LXVIII)

In recent years a number of workers have used " broad " techniques of bacteriological staining with sudan black B, designed to demonstrate non-specifically all the types of fatty material which occur in a great variety of bacteria (Hartman, 1940 ; Burdon, Stokes and Kimbrough, 1942 ; Burdon, 1946). The present note deals with a different application of sudan black—its use as a specific and non-fading stain for the demonstration of tubercle bacilli. This is based on the fact that these are the only organisms which, after staining, have been found to be quite resistant to decolourisation with acetone.

Method

1. The sputum or other material is smeared not too thickly on a slide and is fixed, while still wet, with Carnoy's fluid for half a minute. Heat fixation after drying the smear is a satisfactory but tedious method. Most other fixatives of wet smears are also quite good if not allowed to act for too long. The sudano-phil lipids can be removed by treatment with chloroform or carbon tetrachloride in about half-an-hour, with xylol in about 3 hours and with alcohol in about 8 hours, but they are unaffected by treatment with acetone for over 24 hours. Antiformin concentration methods do not interfere with the staining. Paraffin sections of tissues are always negative, as would be expected.

2. The fixative is drained off, and the slide is half covered with a saturated solution of sudan black B in 70 per cent. alcohol. The stain is lighted and allowed to burn out, and the slide is then swilled with water. The stain is made by adding 3 parts of water to 7 parts of a thoroughly saturated solution of sudan black B in absolute alcohol and filtering the mixture. The diluted stain deteriorates slowly in the course of a few weeks. The stain is efficient over a *p*H range of about 5 to 9, and with various strengths of alcohol between 60 and 80 per cent. Other solvents such as dilutions of acetone, chloroform, dioxane or ethylene glycol are less satisfactory. Prolonged exposure of films to heated sudan solutions tends to stain other organisms, which may then be partially acetone-fast.

3. The gross deposit of stain on both sides of the slide is washed off with acetone, using a drop bottle, and the smear is then differentiated by immersion in a jar of acetone for 2 minutes. Differentiation may be continued for 6 hours without affecting the staining of tubercle bacilli. Immersion for half an hour in acid alcohol, xylol or chloroform does not cause any appreciable loss of stain from these organisms.

4. The smear is rinsed with water, and counterstained with 1 per cent. aqueous solution of pyronin for 10 to 60 seconds. It is then washed quickly with water and dried with blotting paper.

The counterstain should only be strong enough to allow easy focussing. Ziehl-Neelsen cannot be used as a superimposed stain as it removes the Sudan. Furthermore, if it is applied previously, it inhibits the Sudan staining.

Results

Human tubercle bacilli from human lesions (figs. 4-6) are strongly stained black. Sometimes they stain evenly; commonly they have a rather barred appearance, even when Ziehl-Neelsen staining shows no beading. When beading is shown with Ziehl-Neelsen, it is quite different in appearance from the barring in the corresponding sudan-stained organisms. No differences can be recognised between organisms in sputum, urine, cerebrospinal fluid or serous effusions, or in smears made from the thick feltwork of bacilli which grow at the ulcerated surface of lesions such as the lining of tuberculous cavities in the lung.

Human tubercle bacilli from guinea-pig lesions (figs. 1-3) show a considerable range of appearance, even in the same slide. Some stain heavily and evenly, but many show an accentuation of the barred appearance and a variable number have only a slight lipid content. This last type is seen as a thin grey-black outline due to staining of the surface membrane, with perhaps only a single large black mass in the substance of the bacillus.

Bovine tubercle bacilli from lesions in cattle or guinea-pigs have the same staining reaction as human tubercle bacilli in guinea-pigs. No examination has yet been made of organisms, identified as bovine, from human lesions.

Human, bovine and avian tubercle bacilli in culture (on solid media such as Lowenstein-Jensen or Dorset, or in fluid media such as Dubos) are practically unstained by the present method, though they stain normally with Ziehl-Neelsen. They can be stained a feeble brown by prolonged exposure to the sudan solution at 57° C. Some academic interest attaches to the obvious difference between the lipid content of the cultured organisms and that of the organisms in pathological lesions.

The other acid-fast bacilli which have been examined (natural growths of *Myco. smegmatis* and *Myco. lepræ* and cultures of *Myco. phlei*) are not shown by this method. After the application of the sudan stain they are only feebly coloured, and will not withstand more than a few seconds differentiation with acetone.

A variety of other organisms, including Actinomyces, clostridia and the *subtilis* group have been examined by the method described. The only organisms that have been found to stain are certain throat sarcinæ, *C. diphtheriæ mitis* which shows in culture a few small black granules and *C. diphtheriæ gravis*, which occasionally has a dark granule in the broad part of the club forms in culture.

Commentary

For clinical use, the chief advantages of this method as compared with Ziehl-Neelsen are that the time taken from receipt of the sputum to the micro-scopic examination is only about 4 minutes, and that other acid-fast bacilli are not demonstrated. Otherwise there is little to choose between the two methods. The tubercle bacilli are as easy to recognise microscopically; they are black

PLATE LXVIII

SUDAN BLACK STAINING OF TUBERCLE BACILLI

FIG. 1.   FIG. 2.   FIG. 3.

FIGS. 1-3.—Tubercle bacilli from guinea-pig lesions.   ×1700.

FIG. 4.   FIG. 5.   FIG. 6.

FIGS. 4-6.—Tubercle bacilli from human sputum.   ×1700.

instead of red but have a rather more characteristic morphology because of the beading. The two stains are equally reliable. Counts of marked fields, stained first by sudan and subsequently by Ziehl-Neelsen, show that the same number of organisms are demonstrated by both methods. Clinical comparison of the two methods, carried out on a large variety of pathological material during the past 9 years (over 200 specimens), has shown an exact correspondence in results, apart from one urinary deposit in which scanty tubercle bacilli were found with Ziehl-Neelsen but none with sudan.

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RAPID RECURRENCE OF A GIANT-CELL SYNOVIOMA OF TENDON SHEATH

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(PLATES LXIX-LXXI)

Benign giant-cell synovioma and malignant synovioma have generally been regarded as entirely separate entities. A case intermediate between the two in which there was rapid recurrence is here reported.

Clinical history

W. S., a man of 32, first noticed a small swelling on the right hand in November 1947. It was situated on the palmar aspect at the base of the index finger immediately over the metacarpo-phalangeal joint. The swelling rapidly increased in size until in January 1948 it was half an inch in diameter. After this there was no further growth, but he found that the swelling interfered with his work. Never at any time had there been any pain. In May 1948 the tumour, which until then had been thought to be an implantation dermoid, was excised, and it was considered that the whole of the growth had been removed. When the stitches were taken out at the end of a week, the operation site was apparently normal, but two weeks later, when the bandages were again removed, the patient noticed that the swelling had reappeared and was, if anything, larger than before. The tumour continued to increase in size, though mainly in area, the degree of projection remaining about the same. In July, 6 weeks after the original operation, Mr Michael Oldfield amputated the finger, together with the metacarpal bone and surrounding soft tissues. The wound healed quickly and the patient is now, 14 months later, perfectly well, without any sign of further recurrence. There was no history of injury to this particular site, but the patient's occupation, that of cigarette packer, involved forcing packages together by pressure with the palms of the hands opposite the metacarpo-phalangeal joints. On the other hand, he had been so employed for little more than six months when the tumour was first noticed. Prior to this he had spent seven years in the army.

Morbid anatomy

The original tumour (fig. 1) was roughly spherical and measured $1.4 \times 1.0 \times 1.0$ cm. It was encapsulated, and somewhat nodular on the surface, except for one slightly flattened and irregular area which gave the appearance of the tumour having been dissected away here rather than shelled out. The rest of the surface showed the usual patchy brown and yellow pigmentation of the benign synovioma of tendon sheaths. On section the cut surface was greyish-white in colour and slightly translucent, with brown and yellow pigmentation of the lobulated periphery.

The specimen from the second operation consisted of a finger with meta-carpal bone and attached muscles and tendons. There was a swelling on the palmar aspect immediately over the metacarpo-phalangeal joint. On section (fig. 1) there was seen to be a well-circumscribed tumour measuring 1.8×1.5 cm. in diameter and 1 cm. in depth. It was ovoid and slightly concavo-convex, with the longest diameter transverse to the length of the digit. It lay just below the skin and immediately over the flexor tendon sheath, to which it was firmly attached. A slight depression on the deep surface of the tumour corresponded to the position of the flexor tendon. On section the tumour was greyish-white in colour as in the original growth, and again there was brown pigmentation around the periphery, with some bright yellow areas in the more proximal part.

Histology

I. *The primary tumour.* This is a giant-cell synovioma, but much more cellular than usual and with relatively sparse giant cells. The great bulk of the growth is formed of polygonal and spindle cells with varying amounts, though never great, of collagenous interstitial matrix. The polygonal cells tend in places to be aggregated in masses and sometimes they line small clefts or cavities (fig. 2). Elsewhere they are arranged about spaces lined by flattened endothelial cells, apparently blood vessels. Mitotic figures are present, but not in large numbers and on the whole the tumour cells are not particularly anaplastic looking. Multinucleated giant cells of osteoclast type are not numerous but are scattered, usually in groups, about the tumour, sometimes among the cells lining the synovial-like spaces. The lesion is quite vascular and there are small deposits of hæmosiderin apparently within tumour cells and situated particularly around the periphery of the growth. There are also groups of foamy cells (fig. 3), both around the periphery and in the substance of the tumour, and one or two giant cells of Touton type are present.

This growth is more cellular and contains fewer giant cells than is usual in the typical benign giant-cell synovioma, from which, indeed, it deviates rather widely, while still obviously of the same class. It appears to be in process of becoming malignant.

II. *The recurrent tumour.* This is very similar in appearance to the original growth, but in places resembles even more closely than it the benign giant-cell synovioma of tendon sheaths. In some areas there are many typical multi-nucleated giant cells (fig. 4), but, as in the original tumour, most areas contain only a few of these giant cells and are much more cellular and active-looking (fig. 5) than the benign type of growth, and there are abundant mitotic figures (fig. 6). Altogether this tumour is more cellular than the primary growth and shows extensive areas which are both very cellular and devoid of giant cells (fig. 5). The tumour nevertheless is well defined and even encapsulated, and, as in its completely benign counterpart, there are small deposits of hæmosiderin and several patches of foamy-cell accumulation. Occasional synovial clefts are also present.

FIG. 1.—Primary and recurrent tumours on section : primary above. The recurrence shows skin on its upper aspect, with attached tendon sheath below.   ×2.
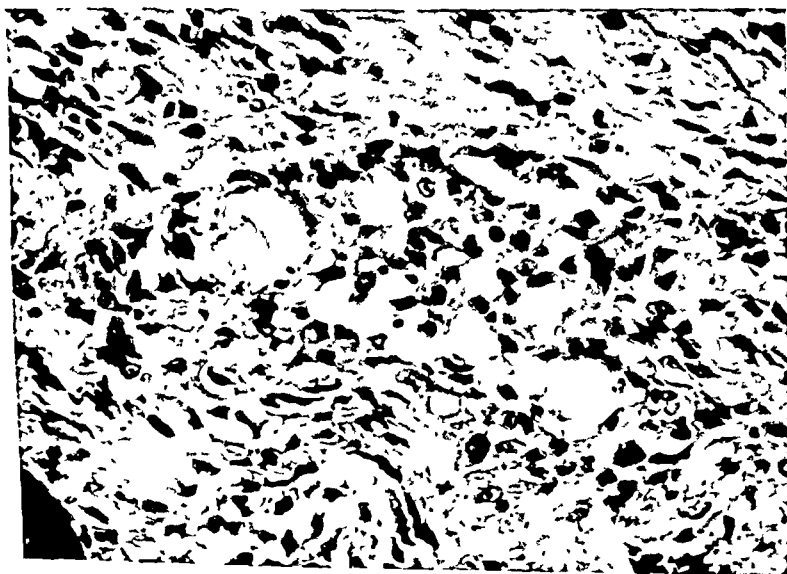

FIG. 2.—Primary tumour, showing early synovial space formation.   ×280.

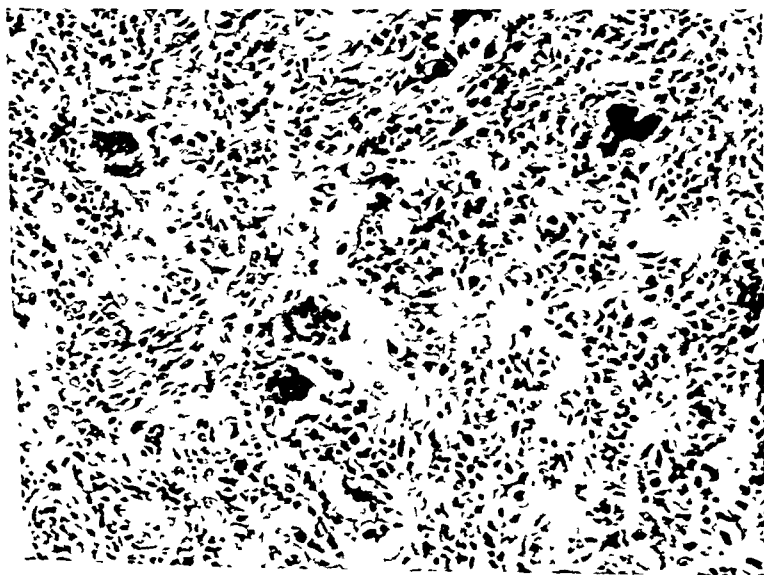FIG. 3.—Primary tumour, showing an aggregation of foamy cells. × 110.

FIG. 4.—Recurrent tumour : area showing the classical features of benign giant-cell synovioma. × 110.

Commentary

The tumour thus presents the characters of a benign giant-cell synovioma which is becoming or has recently become malignant. Structurally it occupies an intermediate position between benign giant-cell and malignant synovioma. The sharply capsulated margin is in striking contrast to what is seen in the frankly malignant tumour, and it is possible that the prognosis may not be quite as bad as the rapid development of the recurrent tumour would suggest. It may well be that an important factor in determining the recurrence was incomplete removal of the original growth where it was attached to the tendon sheath. Peripheral lobulation is a feature of many of these tumours and a further possibility is that a tiny nodule of tumour tissue with only the most tenuous attachment to the main mass may have been left behind. On the other hand, the unusually rapid growth of the recurrence and the deviation from the " normal " histological structure in both primary and secondary tumours point to a change in character of the neoplasm itself.

Berger (1938), in a survey of the literature of xanthomatous giant-cell synovioma, found no indisputably malignant case. He then recorded a tumour which he believed was of this variety. Case V in his series, a male of 50, had a tumour on the anterior aspect of the left thigh. On excision it was found to be invading the quadriceps muscle. Histologically it was described as essentially a fibrosarcoma but with areas closely resembling the classical giant-cell synovioma of joints and tendon sheaths, including giant cells, foamy cells and synovial spaces. Two years after the operation the tumour recurred and was again found to have invaded the muscle. It was again removed locally but there is no mention of further follow-up. The case was thought to have originated in a subfascial serous bursa and was regarded as the malignant counterpart of the benign giant-cell synovioma.

Three years later, De Santo *et al.* (1941), in a study of 16 cases of synovial sarcoma, described three further cases, all associated with the knee joint. In case 11, that of a man of 25, the tumour was described as being formed of atypical cells varying from spindle to polyhedral and having ill-defined cell outlines, with mitoses in moderate numbers. Many areas resembled the giant-cell tumour of tendon sheaths. The tumour recurred and was again excised, but the patient died 2½ years after the first operation from pulmonary metastases. Case 13, that of a male aged 48, and case 14, that of a male aged 46, were considered identical. Both involved the popliteal space, having probably arisen in the semimembranosa tendon sheath. One recurred after four months, the other after eleven months. Following further excision the patients were well two years and one year later respectively. Histologically the tumours were formed of polyhedral and spindle cells with numerous large giant cells in some areas. In the recurrences mitotic figures were said to be fairly numerous, about two per high power field. Synovial spaces, hæmosiderin and foamy cells were present, but foamy cells were rare or absent in the recurrences. The authors considered that these last two tumours constituted a less malignant variety of synovial sarcoma and were more likely to prove locally malignant only.

A further case, as yet unpublished (Stewart, quoted by Willis, 1948), also involved the popliteal space and was invading the muscles.

As is well-known the site of election of the benign giant-cell synovioma is the finger, three out of four in Stewart's (1948) series of twenty-six cases. I have not been able to find a single recorded case of malignant change in one of these tumours situated on the hand. Galloway *et al.* (1940), in a series of seventy cases of benign giant-cell synovioma at the Mayo Clinic, found mitotic figures only on rare occasions and in only twelve of the tumours.

Summary

A synovioma of the hand which recurred within two weeks is described. Microscopically the tumour is considered to be intermediate between the benign giant-cell synovioma and the malignant synovioma. No comparable example has been found in the literature but five cases are recorded (four associated with the knee joint and one in the thigh) as constituting the malignant counterpart of the benign giant-cell synovioma.

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612 . 79 : 576 . 852 . 23 (diphtheroid)

THE PRESENCE OF OLEIC ACID-REQUIRING DIPHTHEROIDS ON HUMAN SKIN

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(PLATES LXXII AND LXXIII)

It is now established that the growth of a variety of micro-organisms is markedly stimulated by oleic acid and other unsaturated fatty acids. The evidence was reviewed by Pollock (1949). In some instances the need for oleic acid is absolute. This stimulating effect of oleic acid for micro-organisms was first described by Fleming (1909) for *Corynebacterium acnes* and, much later, by Benham (1941) for *Pityrosporum ovale*. Both these organisms are known inhabitants of the human integument, frequently found in association with the acne pustule and seborrhœic conditions repectively. The presence of oleic acid or similar fatty compounds, or both, on the human skin (Burtenshaw, 1942) makes it not unlikely that a number of strains of saprophytic or potentially pathogenic micro-organisms requiring unsaturated fatty acids may have their natural habitat there.

In an attempt to discover whether such fatty acid-requiring organisms were likely to be of frequent occurrence on the skin, a throat swab, moistened in broth, was rubbed over an area measuring about 3 × 5 cm. on the extensor surface of the left forearm and inoculated on to a plate of nutrient agar.

Fifty-two apparently different colonies from the ensuing three-day growth were subcultured and isolated. A preliminary test of their need for oleic acid was made by inoculating each strain on to plain nutrient agar, nutrient agar containing 0·2 per cent. activated charcoal (B.D.H.) and nutrient agar with
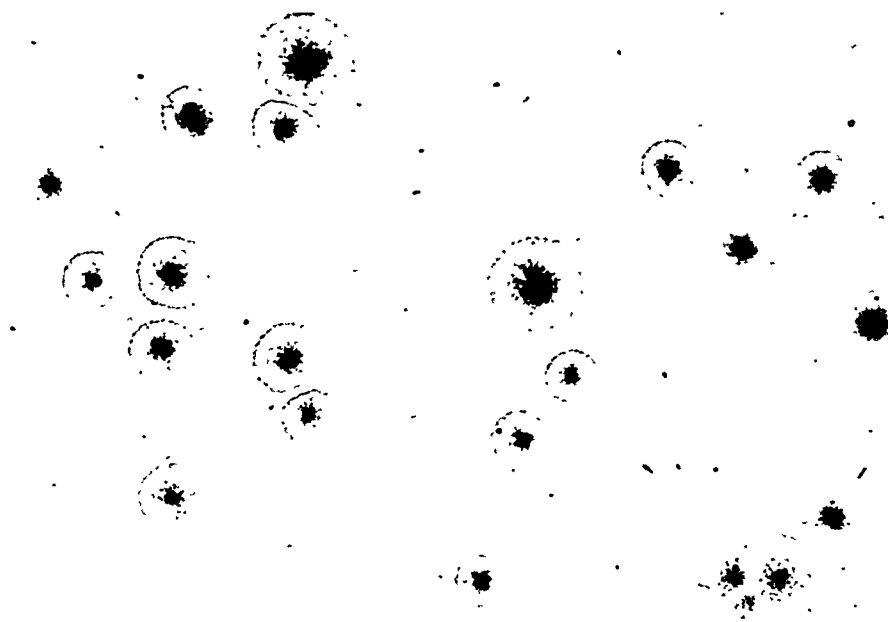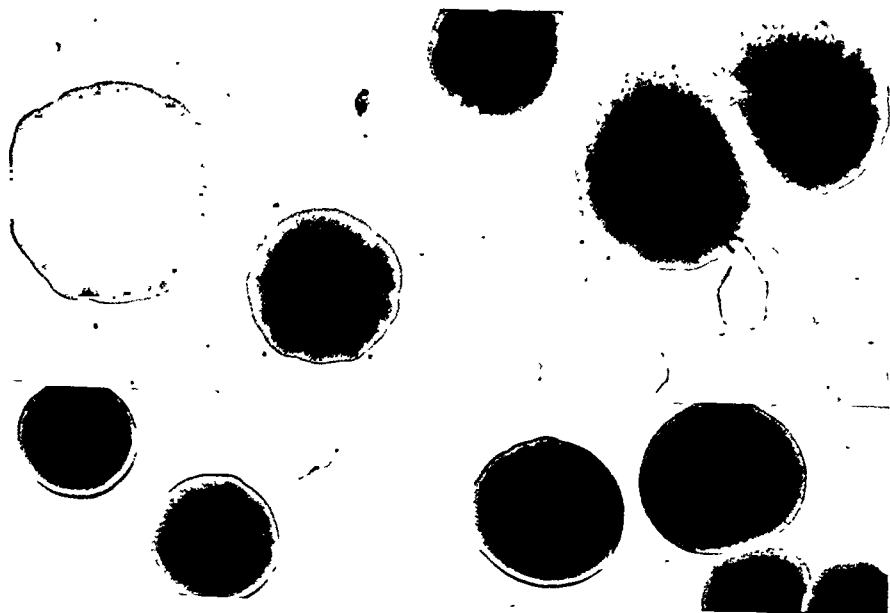
DIPHTHEROIDS ON HUMAN SKIN

FIG. 1 a.

FIG. 1 b.

FIG. 1.—Comparison of size of colonies of the oleic acid-requiring diphtheroid G_{10} grown for 5 days at 37° C. (a) on ordinary tryptic meat agar (T.M.A.), (b) on T.M.A. containing 1 in 25,000 oleic acid.   ×10.
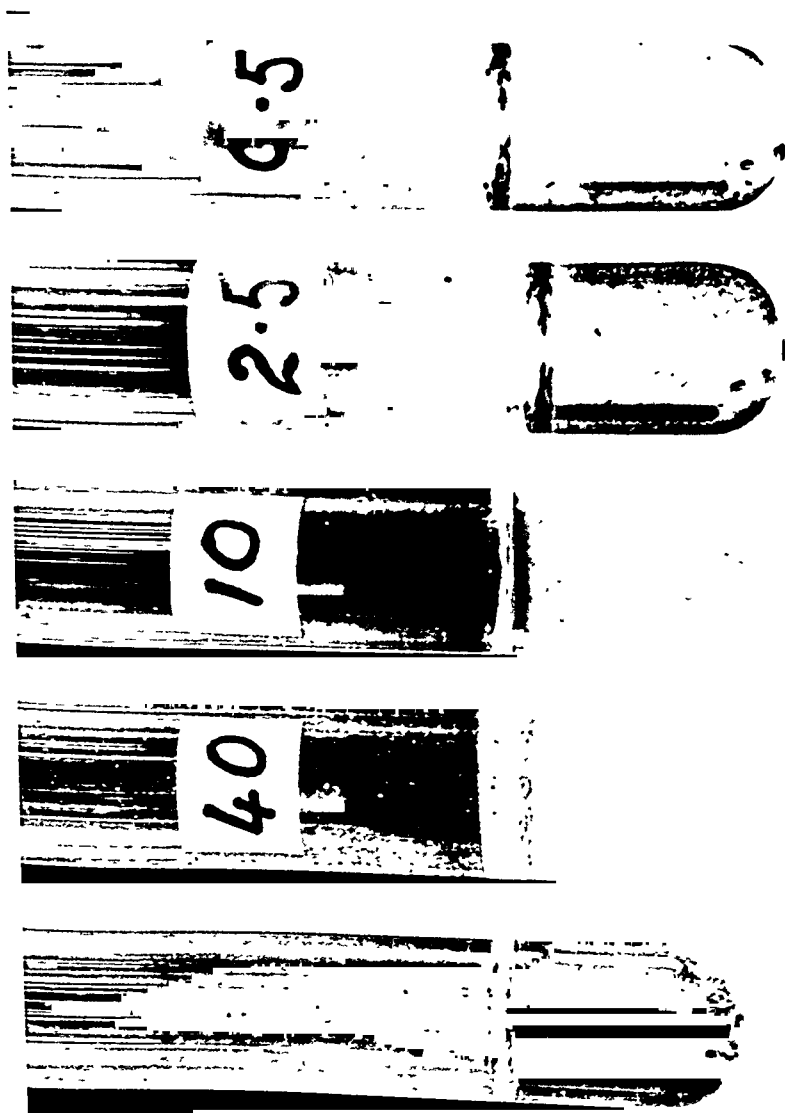
DIPHTHEROIDS ON HUMAN SKIN

FIG. 2.—Graded growth response of the oleic acid-requiring skin diphtheroid C_{10} in casein hydrolysate medium containing increasing concentrations of oleic acid. Figures on tubes give the concentration of added oleic acid in μg. per ml. The first tube, unlabelled, contains no oleic acid and gives no growth. Incubated for 5 days at 37° C.

1 in 25,000 oleic acid. Charcoal is known to absorb the small quantities of free fatty acid present in the tryptic meat base (Pollock, 1947, 1949), so that stimulation by oleic acid combined with inhibition by charcoal was taken as an indication that the organism probably needed oleic acid for growth.

Of 52 strains tested, 5 failed to grow on charcoal agar. All 5 were markedly stimulated by oleic acid (fig. 1). All 5 were inoculated, after the cells had been washed once with distilled water, into a medium containing casein hydro-lysate (Ashe's " vitamin-free ") plus 16 growth factors, and all 5 failed to grow unless oleic acid, specially prepared and purified by Dr G. A. Howard, was added as well : growth as judged by eye, though variable from strain to strain, was roughly proportional to the amount of oleic acid added, from 0·5 to 40 μg. ml. (fig. 2).

Morphologically, all 5 strains were short, Gram-positive, irregularly staining diphtheroid rods, indistinguishable one from another ; but sugar fermentation reactions (table) showed that at least 3 different types were present. It was

TABLE

Sugar fermentation reactions of 5 strains of oleic acid-requiring diphtheroids isolated from human skin

Strain	Glucose	Maltose	Galactose	Sucrose	Fructose	Lactose	Dextrin	Starch	Glycerol
D₁	±	+	+	±	+	+	+	+	—
D₂	+	—	—	—	—	—	—	—	+
D₄	±	—	—	—	—	—	—	—	—
C₃	±	—	—	—	+	—	—	—	±
G₁₀	—	—	—	—	—	—	—	—	—

Tubes contained 1 per cent. of the sugar in Hiss' serum water. Incubation for 3 weeks at 37° C. + = acid formed.

not possible to distinguish between D₂ and C₃ or between D₄ and G₁₀, either by growth characteristics or biochemical reactions, so that these two pairs must provisionally be regarded as identical.

For comparison, representative strains of a number of well-known diphtheroids were tested in a similar way. These included *C. acnes* (from Mr H. Proom, Wellcome Laboratories, Beckenham), *C. xerosis* (N.C.T.C. 7243), *C. murium* (N.C.T.C. 949), *C. ovis* (N.C.T.C. 3451) and *C. pyogenes* (N.C.T.C. 6448), as well as the twelve un-named types isolated and described by Barratt (1924-25) and obtained from the National Collection of Type Cultures by the kindness of Dr S. T. Cowan, the Curator. The Barratt diphtheroid strains were derived from a variety of sources but did not include any isolated from the skin.

All these classified diphtheroids were stimulated or inhibited to some degree by oleic acid ; but, with the exception of *C. acnes*, only one strain, Barratt type III, showed marked stimulation by oleic acid combined with almost complete inhibition by charcoal. This organism grew rapidly in the casein hydrolysate medium in the presence of added oleate, but had not completely lost the ability to synthesise its own oleic acid, since it grew, albeit extremely slowly, in the basal medium alone.

Thus it appears that among diphtheroids absolute dependence on oleic acid is commonest amongst species inhabiting the skin, where there is an abundant supply of the necessary growth factor in an assimilable form. It is possible that oleic acid-requiring diphtheroids, such as those described in this paper, may be of widespread occurrence, presumably as harmless saprophytes of no

pathogenic significance. It should be borne in mind, however, that the powerful self-disinfecting ability of the skin for potential pathogens such as streptococci and staphylococci has been shown by Burtenshaw (1942) and Ricketts, Squire and Topley (personal communication) to be due to the presence of oleic acid derived from the sebaceous secretions. In so far as fatty acid-requiring micro-organisms need and utilise skin oleic acid, they might on occasion be indirectly responsible for a weakening of the natural defences against invading pathogens and so contribute to infection of the body by other organisms.

Summary. Of 52 apparently different microbial colonies isolated from a small area of skin on the forearm, at least 3 biochemically distinct strains were found which had an absolute requirement for oleic acid for growth in a chemically defined medium. All 3 were Gram-positive diphtheroids.

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OBITUARY NOTICES OF DECEASED MEMBERS

Stuart McDonald

27th May 1873–15th November 1948

(PLATE LXXIV)

THE death of Stuart McDonald, emeritus professor of pathology in the University of Durham. occurred suddenly at Edinburgh on 15th November 1948. An original member of our Society, he devoted himself unreservedly to its interests and took an active part in its proceedings and discussions, while at its dinners he added much to the enjoyment of the company by his humorous contributions. He had an attractive and forceful personality, with many characteristic traits. and will long be remembered by all who knew him.

McDonald, the youngest of a large family, was born in 1873 at Castle Douglas, Kirkcudbrightshire, where his father was a prosperous man of business. He was brought up in a lovely countryside, rich in tradition and romance, and from these surroundings he received an impress which affected his mental outlook and tastes throughout his life. He received an excellent school education at Dumfries Academy, which was, and still is, one of the best schools in Scotland and has had among its pupils many who have attained eminence. His great ability soon became manifest ; he won distinctions, and finished his school education by being dux of the school. It is interesting to note that Sir William Wright Smith, the eminent botanist, was dux in the following year. On leaving school, McDonald went to Edinburgh University and, as a preliminary to his medical studies, attended for a year classes in the arts curriculum, chiefly of a literary nature. Thereafter he had a highly successful career as a medical student. distinguishing himself especially in the clinical subjects and graduating M.B., Ch.B. in 1896. At that time there were, and there still are, teachers in the extra-mural medical school whose courses qualified for graduation, and McDonald took not a few of his classes there, always selecting his teacher according to what he considered his merits ; always independent, he never followed the herd.

At an early period McDonald acquired a strong bent towards pathology, and, while still a medical student, gave voluntary assistance to the writer in the pathology department of the Royal Infirmary. He recognised, however, that post-graduate experience in clinical work was indispensable to the successful understanding of the subject, and this experience he obtained in various ways. He first became

house surgeon in the Cumberland Infirmary at Carlisle, where he had Wade, now Sir Henry, as colleague, an association which led to a life-long friendship. For a short time afterwards he had experience of general practice in England, and then became resident pathologist in the Birmingham General Hospital, a post which he held for fully two years. Here he was in charge of the pathological services and had at his disposal a great wealth of pathological material. Thus, being an indefatigable worker, he soon acquired a wide knowledge of his subject and this was further enlarged by his working for a time with Aschoff in Freiburg, from whom he gained, as so many others have done, inspiration as well as valuable instruction.

In 1901, on the appointment of R. F. C. Leith to the chair of pathology in Birmingham, McDonald succeeded him as lecturer in the School of Medicine of the Royal Colleges of Edinburgh. In this post he became responsible for giving complete courses, qualifying for graduation, in both systematic and practical pathology. His duties were onerous, but he had great powers of work and greatly enjoyed teaching. The quality of his instruction soon became recognised ; he proved to be not only an able and successful teacher, but also to have the faculty of imparting some of his own enthusiasm to his students.

McDonald was appointed pathologist to the Edinburgh Royal Hospital for Sick Children and later, on a vacancy occurring, pathologist to the Edinburgh Royal Infirmary. In spite of the heavy routine duties which these posts involved, he prosecuted research on a number of subjects, including an important investigation on epidemic cerebro-spinal meningitis. He made this the subject of the thesis for which, in 1907, he gained the degree of M.D. with honours and a gold medal. In 1900 he became a Member of the Royal College of Physicians of Edinburgh and in 1904 he was elected a Fellow. In 1920 he was admitted to the Fellowship of the Royal Society of Edinburgh.

His departure from Edinburgh in 1909 on appointment to the staff of the College of Medicine at Newcastle upon Tyne was much regretted by his many colleagues and friends in Scotland, and he left behind him a high reputation both as a pathologist and as a man.

R. M.

When Stuart McDonald came to Newcastle in 1909 he was 36 years of age and already a pathologist of established reputation. He was the first whole-time pathologist in the Newcastle School, and, though actually appointed as lecturer, was almost at once elevated to a professorship in recognition of his standing as well as the importance of his subject.

It must be admitted that there was not much of a department but there was a great tradition in morbid anatomy, linked especially with the names of Byrom Bramwell and David Drummond, and there was a very good pathological collection in the museum. But McDonald was in no way dismayed and threw himself into the work

Stuart McDonald

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of organisation with great enthusiasm, and it was not long before his efforts yielded results.   He soon reorganised and enlarged the laboratory and class rooms and gathered together a staff who helped him most loyally.   His efforts were greatly aided by his early appointment as pathologist to the Royal Victoria Infirmary, for that opened up a tremendous field, which he was not slow to cultivate.   From the outset the importance of his subject in the School curriculum was much enhanced and his lectures and laboratory teaching were greatly appreciated.   McDonald co-operated well with the clinicians and throughout the whole of his tenure of office his relations with the staff of the Infirmary were cordial and of the greatest benefit to the work of the hospital and to the status of the School.

Mainly a morbid anatomist, he was particularly keen on the histological side and used to take great pains to render his reports both helpful and interesting.   He would take a lot of trouble discussing points concerning the pathological interpretations, and these informal consultations were of the greatest value to the younger clinicians. From the outset he inculcated a great lesson by insisting that whenever possible the slides should be studied side by side with the gross specimen.

In his earlier days in Newcastle, McDonald did good work on acute atrophy of the liver and on lung conditions associated with industrial disease.   He also worked on meningococcus infections, which involved much experimental work on monkeys.   It was in this connection that I recall his insisting that in pathological problems it was necessary to " get back to the soil," for he had constantly in mind the reactions of the living body to pathological invasion.

From time to time he also undertook medico-legal work, when his care and attention to detail ensured that his opinion was much valued in legal circles and he was regarded as a reliable expert witness.

He was a great encourager of young workers and in 1929 no less than three of his assistants obtained Rockefeller Fellowships.

In the 1914-18 war McDonald turned to and did some clinical work in the first Northern General Hospital, which I think he very much enjoyed.   Certainly he showed himself a good clinician, his early experience in hospital and in a country practice standing him in good stead, for he would remember and recount many of the lessons which had made such an impression on him in those early years.

Coming from a great medical school like Edinburgh, it is not surprising that he took his teaching duties seriously.   This was very much to our advantage in Newcastle and his efforts were much appreciated.   McDonald was a good examiner and his services were much sought after in that capacity.   He had great sympathy with the shy or reluctant student and a great sense of fairness.   His object was always to find out what the student knew and only under provocation would he plumb the wells of ignorance.

At quite an early period of his tenure he arranged a meeting of

the Pathological Society in Newcastle. It was in July 1912 and I well recall how he roped many of us in to help and the zest with which he made preparations for what turned out to be a very successful and memorable gathering. A similar meeting was held in 1924 but that was rather more specialised. I really believe that one of the best things he did in Newcastle was to start the Pathological Club in 1910, just the year after his appointment. This drew its members from all the workers about the School and included some of the more enthusiastic medical practitioners, with a not inconsiderable number, be it said, of those from his own Alma Mater. For some years the Club was a high light in the School, and its meetings, held in the evening after dinner and sometimes carried on until a late hour—never too late for McDonald—were greatly enjoyed by all who could manage to be present. McDonald was always the guiding spirit and I am sure that the venture did much to help the younger people and to keep up the interest of the more senior.

As time went on and the School developed, problems of organisation and management came more and more to the fore and McDonald was drawn into the vortex. He was elected to the Council of the College, was made Chairman of the Academic Board and later a member of Senate. In 1927, on the retirement of Professor Howden, he became Dean of the School and there is no doubt he was most successful in this capacity, but I think we must admit that his activities in this direction were only carried out with considerable and regrettable loss to his own Department. As Dean he took the greatest personal interest in the students and many young men must owe him a lasting debt of gratitude for the care and patience with which he endeavoured to help them in their individual problems, even when their difficulties extended far beyond the purview of his own department. But from the beginning he had been interested in the student body, who held him in affectionate regard. He had quite a flair for associating with young folk and managed them in a remarkable way. Possibly his interest in athletics played some part in this success, which reached its peak when he became president of the Rugby Football Club. During that period he never missed a match at home or away. His advice to the players and his personal interest in their difficulties contributed to their great success during a remarkable season.

McDonald also took a keen interest in University life generally and grew to be very fond of Durham. He loved to attend academic and social functions in the beautiful setting which the University precincts provide in that fine old city, and the writer has vivid memories of his enthusiasm as we motored the sixteen miles home on fine June evenings after some function at the Castle.

He was a first-rate companion and soon came to be recognised as a great raconteur. Some of his stories were really priceless and this aspect of his personality became quite a tradition among those who were privileged to hear him at his best. He really had a great love

for his fellows and responded well to the kindly approach, but he could be taciturn and was not always too tactful with those who disagreed with him.

He was intensely fond of the country and the open air, and fishing was his great relaxation. It is perhaps not surprising that a man who had suffered great sorrow and had to endure serious provocation was liable to periods of despondency, but in the worst times he found comfort in the open air and especially if his excursion took him northwards towards familiar surroundings in his own homeland over the Border. Though not a musician, he loved music and certain types made an especial appeal to him, for there was something of the mystic in his character. When harassed and worried he found relief in listening to some of his favourite records with the lights out. Poetry also made a strong appeal and almost our last correspondence concerned the life story of the young Northumbrian poetess, Ada Smith, whose " In City Streets " he regarded as very fine and often quoted, I think because her lines echoed a recurrent sentiment in his own make-up :—

> " My eyes an ache to see the brown burns flowing,
> Through the peaty soil and tinkling heather bells."

The early death of his young wife was a stunning blow : it inflicted a psychological injury from which he was slow to recover. Gradually the realisation of his responsibility for his only child created a new outlook and he found consolation and awakening pleasure in the upbringing of his son. The success of young Stuart academically and in his chosen career in pathology, his notable war service and appointment to the St Andrews chair of pathology lightened sad years of failing health and diminishing physical powers, but his tragic death in 1947 was a knock-out blow, the results of which his father carried to the grave.

McDonald retired from the chair and left Newcastle in 1938.

G. G. T.

If one views McDonald's original work in pathology as a whole, one may say that it was practically confined to morbid anatomy and histology. The only exception to this was his experimental work on epidemic cerebro-spinal meningitis, in which he studied the transmission of the disease to monkeys. This work, published in two papers in 1907, was of a comprehensive nature, and it is interesting to note how closely many of his conclusions are in conformity with what has since been firmly established. The list of his contributions includes papers on a variety of subjects. He took a special interest in the more acute lesions of the liver and his paper on acute yellow atrophy in syphilitic subjects treated with salvarsan is important and suggestive. He was the first to observe the characteristic " bodies " in the lung lesions of asbestosis, an observation which proved to be most valuable in the elucidation of the disease. Other papers are mainly on individual

cases and need not be referred to in detail. All are characterised by careful and accurate observation and balanced judgment.

It should be noted that McDonald liked to work in collaboration with others and not a few of his papers are joint productions. This was in keeping with his faculty of stimulating his students in research, and he had very real pleasure in any success obtained by them. His teaching and extensive routine hospital duties, however, gave him little time for sustained research on a particular subject. His medico-legal work too made heavy inroads on his time, for he always maintained a high standard in these investigations. He was in fact eminently conscientious, and made it a rule never to state in evidence what he would not be prepared to present to a medical society. The organisation of hospital pathological services and the arrangement of the medical curriculum were two other subjects to which he gave both time and thought.

McDonald was a man of scrupulous uprightness and great kindliness, with a friendly interest in the wellbeing of those around him. In all his activities there was one outstanding feature—his zest. This was seen both in his pathological work and in his recreations. He was enthusiastic in outdoor sports, and especially in fishing, golf, curling and bowling. It was from the first of these, however, that he derived the greatest pleasure, and he was as expert as he was enthusiastic. Many were the delightful angling outings that he had in various parts of Scotland, and also in Norway and Iceland, often in company with his son Stuart. From each of these he would bring back strange tales, as well as curious facts in natural history for which he was ever on the outlook. And he looked the countryman, with his big frame and rubicund countenance, his curly hair and the kindly twinkle of his eyes behind (or beneath!) his spectacles. His delight in nature was ardent and enhanced by knowledge of all her moods and vagaries, and his life in the country in later years gave him enviable opportunities of study and appreciation. His love of flowers amounted almost to adoration, and the little wayside blossom found a place in his heart along with the rose in its full and glorious beauty.

His zest was seen in other departments also. He was a great reader, with full appreciation of what was good in literature and decided tastes as to favourite authors. He was devoted to poetry, notably that of Keats, and was himself a writer of characteristic verse of no small merit. His memory was remarkable. He had a great liking too for a good picture, and excellent judgment therein. It is not surprising that these tastes and qualities made him a conversationalist of unusual attractiveness: a " night with Mac " was always a thing to be looked forward to by his friends and to be remembered afterwards.

His vitality and keenness, however, were not uniformly maintained. He was temperamental—there were times when he would cut himself off from his associates and become low spirited. Such moods came

to be understood and reckoned with by his friends, but with others they sometimes led to misunderstanding. It is important for an understanding of McDonald's personality that these facts should be recognised.

McDonald had in his life heavy and painful losses, some of which have been already referred to. In particular there was the death of his charming and gifted son, which quite prostrated him for a time. In his later years he had also heavy disabilities from his own state of health. Cataract in both eyes appeared, and operation on one eye revealed also the presence of choroiditis of an intractable nature. There was progressive loss of vision and he became quite unable to read. He had also to face the disability of prostatic and later still of myocardial disease. In spite of these heavy trials, McDonald had for some months before the end regained some of his old mental vitality, and even the capacity for enjoying things outside himself. Compelled by the state of his health to give up his house at Broughton. he came to live with his nieces in Edinburgh, and at his club there he derived intense pleasure from occasional meetings with a small circle of friends at lunch or dinner. At these, as in the old days, he was always the life and soul of the party.

He died in his sleep, which is as he would have wished.   R. M.

ROBERT MUIR
G. GREY TURNER

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James McIntosh
1882–1948

(PLATE LXXV)

JAMES MCINTOSH was born in Aberdeen on 6th September 1882. He was educated at Robert Gordon's College and at Aberdeen University, where he graduated M.B., Ch.B. in 1905 and gained an Alexander Anderson Scholarship. In 1906 he went to Paris for the bacteriological course at the Pasteur Institute and carried out research with Levaditi. Returning to Aberdeen in 1908, he was awarded the M.D. with highest honours. In the autumn of 1908 (not, as stated in the *Lancet* and *British Medical Journal*, in 1910) he joined his fellow townsman William Bulloch at the London Hospital, holding first a Carnegie research scholarship and later, in 1910, a Grocers' scholarship. Here he remained until 1920, when he became professor of pathology in the University of London and director of the Bland-Sutton Institute of the Middlesex Hospital in succession to C. H. Browning. This appointment he held for the rest of his life.

McIntosh served as President of the Pathological Section of the Royal Society of Medicine, Treasurer of the Pathological Society of Great Britain and Ireland from 1930 until his death, Chairman of the London University Board of Studies in Pathology, President of the Institute of Medical Laboratory Technology and examiner to the Universities of London, Cambridge and Manchester and to the Conjoint Board.

In the list of McIntosh's published work it will be seen that a number of his early papers appeared in foreign journals. At that time there was a serious dearth of pathological journals published in this country and deliberations took place between J. A. Murray, W. E. Gye and McIntosh on the question of starting a new journal. The present writer was sounded by McIntosh and in due course a group of younger pathologists undertook the financial risk of founding the *British Journal of Experimental Pathology.* Throughout his life McIntosh retained a close interest in the new journal and in it he published much of his research work. Latterly, after the journal had been turned into a company limited by guarantee, he became the first vice-president.

McIntosh, who was a bachelor, died in Aberdeen on 5th April 1948.

Notes on McIntosh's personality appeared in the *Lancet* and *British Medical Journal* of 17th April 1948. The present writer was intimately associated with him from 1908 to 1916 and from 1934 to 1940 and, of course, less intimately in the intervals. It is common knowledge that some found him " difficult." So far as concerned the present writer, an Englishman, who had long association with William Bulloch and could make some allowance for national characteristics, no " difficulty " was experienced. He was a pleasant, interesting and loyal colleague and companion.

His junior, R. W. Scarff, has written of him that :—" He possessed the quick temper and generous nature that so often seem to go together and so often seem to inspire loyalty. Intolerant of inaccurate or slipshod work he would show great kindness and understanding in the difficulties of others. He had the power of infecting his colleagues with his own enthusiasm and his wise and successful guidance of the Bland-Sutton Institute of Pathology over so many years was in no small part due to his knowledge of just how much help his assistants needed in their work without in any way limiting their freedom of thought."

RESEARCH WORK

McIntosh's activities in medical research may be described under the major groups in which he became interested. Of these the first and not the least important was syphilis.

Syphilis

It will be remembered that prior to 1903 little was known about the pathogenesis of syphilis. It was not a subject which came into the field of experimental pathology at all. In 1903 Schaudinn discovered *Sp. pallida* ; in the same year Metchnikoff and Roux transmitted the disease by inoculation ; in 1906 Wassermann, Neisser and Bruck described the " Wassermann reaction " and Landsteiner and Mucha the dark-ground method of demonstrating the

James Whitlock

spirochæte; in 1909 Ehrlich and Hata discovered "606"; in 1910 the first clinical trials were published and the preparation was put on the market. Thus in seven years the causation, diagnosis and treatment of this obscure disease were established and during part of this time McIntosh was working in Metchnikoff's laboratory with Levaditi, the discoverer of a practical method of spirochæte staining. After his return to England full of this subject he worked with the present writer in Bulloch's laboratory at the London Hospital. A book published in collaboration in 1911 showed how syphilis could now be dealt with "from the modern standpoint".

McIntosh's first work on syphilis was with Levaditi, the results of which were published in 1907. A number of unsuccessful attempts had been made to cultivate *Spirochæta pallida*, now known as *Treponema pallidum*, particularly in collodion sacs in the rabbit's peritoneum, and Levaditi and McIntosh tried the monkey's peritoneum. A capsule containing chancre material from a monkey was placed in the peritoneum of another monkey which was simultaneously inoculated in the eyebrow. Twenty-three days later the eyebrow developed a chancre and the peritoneum was then opened. The capsule was found to contain an impure culture of a spirochæte resembling *T. pallidum.* They had no difficulty in passaging this first culture through rabbits, but it was always mixed with anærobes. Attempts to obtain cultures *in vitro* failed. Whether the spirochæte of Levaditi and McIntosh was indeed the spirochæte of syphilis cannot now be decided. They were unable to demonstrate any pathogenicity in the mixed cultures. Levaditi had already described his staining method for spirochætes in tissues and McIntosh took up and developed a lasting interest in histology. He took his M.D. (Aberdeen) on a thesis describing the distribution of *S. pallida* in congenital syphilis, and in 1909 published a paper on the presence of this organism in the ovary of an infant which died a few days after birth with acute generalised syphilis. He concluded from this that the possibility of the direct transmission of syphilis to the offspring through the maternal ovum could not be excluded. It does not seem, however, that his data had direct bearing upon this matter.

In Paris, also, McIntosh had become familiar with the early work on the Wassermann reaction. He had published in 1909 a paper on the clinical aspects of this reaction. He made an important contribution to knowledge when he demonstrated that a positive Wassermann reaction could be taken to be a sign of active syphilis. The Wassermann-reacting antibody differed from ordinary serum antibodies in this respect. He found that trypanosome-infected rats developed both specific antibodies and Wassermann-reacting bodies. On treatment with atoxyl, leading to disappearance of trypanosomes, the Wassermann-reacting bodies disappeared from the serum, while the specific antibodies were unaffected. Similarly, he found that cure of syphilis with "606" led to disappearance of the positive

Wassermann reaction. In due course he published several papers on the practical aspects of the reaction, and in 1912 introduced the formula for a cholesterolised alcoholic heart extract which has since become the standard antigen used in the test in place of alcoholic extracts of congenital syphilitic liver. Later he undertook a comparison between the results of the Wassermann reaction and of the flocculation reaction of Dreyer and Ward, and proposed a method for converting Wassermann quantitative results to " units " on the lines of Dreyer's system.

McIntosh was also early involved in the introduction of salvarsan. In 1910 he published a paper on the effect of " 606 " in relapsing fever in rats. A year later, clinical trials in syphilis had proceeded far enough to permit publication, and he then for the first time collaborated with the present writer.* In the course of intravenous treatment it was found that severe febrile reactions occurred (1912). These had been ascribed to a " salt " effect, but it was shown that dead bacteria which had grown in the distilled water were the real cause. Incidentally, it was shown that salt solution containing dead bacteria had a remarkable curative effect on some conditions, a fact which was re-discovered later under the title " protein shock therapy."

In 1913 was published the first of a series of papers on syphilis of the nervous system in which Sir Henry Head took a great interest.   . The expression " parasyphilis " was then in use to describe such diseases as general paralysis and tabes, and it was far from being generally accepted that these were direct manifestations of syphilis and not degenerative sequelæ of syphilis or indeed of other conditions. It may certainly be claimed that McIntosh's work accelerated acceptance of the view that they were normal syphilitic processes occurring in the nervous system, not differing from syphilitic processes elsewhere. Sometimes the chief action was exerted on the parenchyma (nerve cells), sometimes on the vascular tissues.

When salvarsan treatment became general it was soon found that the parenchymatous forms of syphilis of the central nervous system often failed to respond to the drug. It was shown (1914) that when salvarsan was injected into man or animals, arsenic could be found in all parts of the body except the brain. It appeared that there was an anatomical barrier between the blood vessels and the nervous tissues through which salvarsan could not pass. Experiments (1916) were undertaken to " carry " the arsenic in combination with a dye capable of passing this barrier but these were abandoned on the outbreak of the first German war.

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* At this time McIntosh was a research worker, the present writer a routinist. The latter, though a year older, was of course less experienced in research.

Virus diseases

McIntosh's work on cerebral syphilis had developed his interest in other infections associated with changes in the nervous system. In 1913 he published a paper with Hubert Turnbull describing the transmission for the first time of poliomyelitis from English cases to monkeys. Again in 1920 and with the same collaborator he transmitted the virus of encephalitis lethargica to the monkey from man. Previous claims by foreign workers were considered to be open to criticism, but in his own work the disease was reproduced with all the fundamental clinical and pathological manifestations. Subsequently this strain was passaged twice both in monkeys and in rabbits and one control monkey acquired the disease spontaneously. Later, improved technique gave such constant results in rabbits infected directly from human material that inoculation could be used for diagnostic purposes.

As early as 1912 Turnbull had noted the occurrence of encephalomyelitis in recently vaccinated subjects and watch was kept for further cases. In 1926 seven cases were reported on by Turnbull and McIntosh. Vaccinia virus was found to be present in the brain and cord and this was the only virus found. The pathological and clinical picture differed from that of encephalitis lethargica and poliomyelitis but resembled that of variolous encephalitis. Further, a comparable condition was produced in animals by inoculation with vaccine lymph. The possibility of producing fatal encephalitis by vaccination naturally was disturbing to the Ministry of Health, and the matter was investigated by a committee which included McIntosh. In due course the committee issued a majority report, leaving McIntosh in a minority of one. Anyone who reads Turnbull and McIntosh's paper at the present time will be surprised to learn that the Committee " attached no credence " to the hypothesis that the encephalitis was due to vaccination. He will note, however, that the report is so full of reservations as to leave it with little or no meaning, but the underlying suggestion is clearly damaging to Turnbull and McIntosh. The report of the committee was so little decisive that another committee was formed three years later from which McIntosh was excluded. This committee went so far as to acquit " vaccinia virus of being the *sole* cause of this complication " but was " unable to exonerate vaccination from playing some part in its causation." Drafted in other words, suitable for plain people, the report might readily be accepted as a commendation of Turnbull and McIntosh.

Justifiable indignation did not prevent McIntosh from pursuing the matter. With Scarff he was able to show that the lesions in animals were produced solely by vaccinia virus without the intervention of some other hypothetical virus which naturally could not be proved to be absent by the committee. Further, histological

examination revealed constant differences between the encephalo-myelitic lesions caused by different viruses.

McIntosh's investigation of the Great Epidemic of influenza did not lead him to support the idea, then developing, of a virus as a causative agent. He was able to reproduce conditions in animals indistinguishable from influenza by means of filtrates of the influenza bacillus, presumably toxins, while with filtrates of nasal washings he produced no ill effects. The production of antibodies to *B. influenzæ* in patients and the therapeutic effects of convalescent sera indicated that an infection by *B. influenzæ* certainly took place. Much later (1933), after the publication of Shope's papers on swine influenza, he took up this work again. He made anærobic liquid cultures from filtrates of human influenza lungs, and after a number of days found a diffuse haziness in serum broth. No organisms were seen by staining or darkground illumination but the haziness was propagated in series. He described the haziness as a " microbe ". The microbes passed a filter and centrifuged deposits stained by Fontana's method showed fine particles. The microbe survived boiling for " several minutes ". Inoculation produced no effect and nasal instillation in rabbits was ineffective. These animals, however, showed lung hæmorrhages after being killed. It will be seen that this short paper is in some respects unconvincing.

Still later (1937), stimulated by Wilson Smith, Andrewes and Laidlaw, he returned to the subject with Selbie. He propagated further strains of haziness by the same methods and this time instilled it into the nose of a ferret. This animal developed fever and the lungs were found to be hæmorrhagic. Filtrates from the lungs produced the same effect in other ferrets. The original ferret material was also effective in mice and could be propagated in series, and eventually the disease was transmitted to a monkey. Convalescent influenza sera and anti-influenza sera (Smith, Andrewes and Laidlaw) were to some extent protective. McIntosh now became convinced of the role of a virus in influenza though, no doubt, still retaining some interest in the influenza bacillus.

Tumours

McIntosh became involved in the study of tumours from his contacts with J. A. Murray and W. E. Gye at the Imperial Cancer Research Fund. In 1910 there was some discussion on the significance of spirochætes in ulcerated carcinomatous lesions or mouse tumours propagated originally from material raised by the Imperial Cancer Research Fund. This fresh material stained by Levaditi's method failed to show spirochætes and thus these organisms could not be implicated in the causation of cancer.

He also took up the subject of the Rous sarcoma of fowls in which a filterable virus was coming to be recognised as the exc··· ͺcause.

The question was bound up with a possible virus cause of mammalian cancer. McIntosh supported the role of a virus in sarcoma but was dubious about the evidence in mammalian cancer.

Failure to obtain " takes " with filtrates of Rous tumours had led some to doubt whether a virus was concerned. Baker and McIntosh (1927) thought that the variation might be due to the physical state of the material to be filtered. They therefore tried tryptic digestion. It was found that trypsin at an acid reaction much increased the infectivity of filtrates, but at an alkaline reaction infectivity was reduced. On the other hand without trypsin an acid reaction was destructive and an alkaline not. Trypsin inactivated by heat had no action. The nature of the acid-trypsin effect was not discovered but the present writer is informed that the phenomena were probably due to the HCN used as a preservative for the trypsin.

McIntosh then turned to tar tumours in fowls. He was probably the first to show that tar tumours of a sarcomatous type were filterable, i.e. that cell-free filtrates of tar-induced tumours could produce similar tumours in series. Thus, tar tumours were in every way comparable with the Rous sarcoma and must equally be assumed to have a virus causation. It must be stated, however, that during his life the production of tumours by filtrates of tar tumours was not confirmed. Nevertheless, the present writer is informed that recent work seems likely to develop results which would have gained McIntosh's full approbation. McIntosh was at least able to show that the agents of Rous and tar sarcomata were particulate, since they could be removed from a suspension by centrifugation at 40,000-60,000 r.p.m., which treatment also deposited bacterial viruses. These general results were confirmed later with Selbie. Some tumours formed in fowls by tar derivatives are filterable, others are not, but can be propagated from suspensions. Trypsin has already been mentioned as capable of increasing the activity of filtrates of Rous sarcoma. The same was found with tar tumours, though there was some variation. In general, it seemed that the action of trypsin was proteolytic. (See, however, the previous remark on HCN.)

Chemotherapy

Work with atoxyl, salvarsan, sulpha drugs, penicillin and other agents interested McIntosh at various stages throughout his career. His second paper, in 1907, had to do with atoxyl, his last, in 1946, with penicillin.

An interesting paper with Levaditi in 1910 shows the beginnings of ideas which even to-day are not generally recognised. They described the fluctuation in the trypanosome content in the blood of rats infected with nagana. After the " crisis ", the blood of these animals is rich in trypanolytic antibodies working both *in vivo* and *in vitro*. When a relapse takes place the trypanosomes are resistant

to these antibodies. They showed by experiments *in vitro* that this was not a question of an alteration in the trypanosomes induced by the antibodies, but of a selection of trypanosomes which were naturally immune.

Curiously enough, many years later (1943) when working on the resistance of Staphylococcus to drugs, McIntosh used his method with Levaditi but described the " principle (as) being to train organisms step by step to survive increasing concentrations of the drug." His earlier idea would probably be preferred at the present day.

During the second German war the work of Florey at Oxford and his own work with Whitby naturally led McIntosh to continue, with collaborators, clinical tests of sulpha drugs and penicillin, and he also studied various flavines. All these papers were of great practical interest.

Miscellaneous

In the course of his career McIntosh touched on a number of other subjects. His first paper was on tuberculosis, and, with colleagues, he made an examination of dental caries. A lactic acid bacillus was isolated and given generic status under the name *B. odontolyticus acidophilus* with the implication that this organism is concerned specifically with the production of dental caries. This claim has not received general acceptance.

War bacteriology

McIntosh's career was affected by both German wars. In the first he became involved in more or less routine matters such as the preoccupation with meningococcus carriers. He was, however, able to break new ground among the anærobes. A modification of the idea of Laidlaw produced the " McIntosh and Fildes jar " which has since become in various forms a piece of standard equipment. It is difficult to realise that in those days a pure culture of many anærobes was the exception and McIntosh's Medical Research Council report on the classification of anærobes (1917) was of great importance.

On the outbreak of the first war it was found that the failure of German supplies made it necessary for British bacteriologists to think for themselves. Great activity was centred on culture media and McIntosh became interested in the estimation of pH in place of titratable acidity. This work was followed up by the Pathological Committee of the Medical Research Council. A further and more elaborate paper on the same subject appeared in the first issue of the *British Journal of Experimental Pathology* in 1920.

The second war found him head of his Institute and involved in much administration. Nevertheless, with willing helpers he continued to produce a number of papers on anærobes, chemotherapy and allied subjects from his temporary war station.

The writer has had the benefit of advice from W. E. Gye and R. W. Scarff in the preparation of this notice. The bibliography was compiled by R. W. Scarff.

PAUL FILDES

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William Frederic Harvey
1873–1948

(Plate LXXVI)

W. F. Harvey died in Edinburgh on 11th September 1948, at the age of 76. Although he had retired in 1946 from his official position in the laboratory of the Royal College of Physicians, he still frequented his old haunts and was always eager to discuss pathological problems, especially of tumour growth or hæmatology, until a month or two before the end, when decreasing strength inhibited his movements but not his enthusiasm or geniality.

His life's work falls naturally into two periods, the first in India, the second in Edinburgh. Throughout it was characterised by ability, enthusiasm, perseverance, and a kindly regard for his fellow men, for no one who knew Harvey could fail to be attracted by him, while to work with him was to love him.

His father was Principal of the Maharajah's College, Travandrum, Travancore, India, where Harvey was born, but he came home for his education, first at Dollar Academy and then at Edinburgh University, where he graduated in medicine with first class honours in 1897. He was house physician to Professor Greenfield in the winter of 1897-98, and possibly that association may have inclined his mind to the study of pathology, for, after entering the Indian Medical Service in 1899, he soon interested himself in the various problems clamouring for research. Early in his service he met Almroth Wright, who was then at Netley, and later, while on leave, he worked in Wright's laboratory at St Mary's. The influence of that contact may be traced in the many improvements which Harvey, now in 1902 Deputy Sanitary Commissioner in the Punjab, made in the manufacture and distribution of vaccine lymph and in the introduction of glycerinated calf lymph into that Province. This was a time of great scientific advance in the Indian Medical Service, and Harvey was prominent in that company of distinguished research workers which included such men as Bannerman, Semple, Lamb, Christophers, Glen Liston, S. P. James and A. G. McKendrick. He was amongst the first group of officers to join the Indian Medical Research Department, being appointed assistant director of the Pasteur Institute of India. In 1905 he succeeded Lamb as director, McKendrick became his assistant and they worked together on the prevention of rabies. McKendrick became his life-long friend, and their association was renewed again in Edinburgh when McKendrick became Superintendent of the Royal College of Physicians' laboratory, with Harvey as histologist.

In 1910 he married Miss Jean Sutherland. They had two children, a son, who is also in the medical profession, and a daughter.

PLATE LXXVI

In 1911 Harvey was appointed director of the Central Research Institute at Kasauli, where he and his colleagues became immersed in bacteriological and immunological problems. His list of publications during this period shows both the variety of his work and the energy which he put into it : many papers were published in the *Indian Journal of Medical Research*, of which he was the first editor when it was founded in 1913. The 1914-18 war broke into his research work, for in 1916 he was recalled for military service and sent to Mesopotamia as an A.D.M.S. (Sanitation). For his organising work there he was mentioned in despatches. An attack of paratyphoid fever resulted in his being invalided to India, later to return to his post at Kasauli where he worked until he retired from the Indian Medical Service in 1925 with the rank of Lieut.-Colonel. For his work in India he received the C.I.E. in 1921.

For Harvey to retire meant merely a change of scene and work, and he returned to settle in Edinburgh and to succeed James W. Dawson. in 1927, as histopathologist to the laboratory of the Royal College of Physicians. So began the second period of his professional career. In his new work he displayed the same energy and thorough-ness that had marked his work in India. It was of a different type, with different problems, but Harvey mastered the difficulties and enriched the literature of his subject by his many additions. The histology of tumours intrigued him, and he began to build up his collection of tumours, carefully indexed and documented, which has been of the greatest help to all interested in this subject. It was his carefully tended child which had grown to the lusty manhood of over 20,000 specimens before he retired. Much of his work on this subject was published in collaboration with Mrs E. K. Dawson and J. R. M. Innes in the " Debatable Tumours " series of papers. For 20 years he laboured happily in his little, low room in the laboratory, always pleased to greet a colleague or an interested visitor, always willing to give useful advice or help in a problem. Not only did his work take form in many papers, it also brought him the Lister and Freeland-Barbour Fellowships and, in 1946, the Cullen prize of the Royal College of Physicians of Edinburgh, in which year he also became a Vice-President of the Royal Society of Edinburgh. During the second world war, he had heavy routine reporting duties in the laboratory, and for a time he acted as superintendent after A. G. McKendrick's death. 1946 saw his official retirement but he still continued working and writing, freed from a routine that had become a task, heavy, but faithfully performed.

The list of his publications, by himself or in collaboration, gives some measure of the energy and industry of the man, but he had many other interests. He was essentially a sociable man, a keen tennis player in his earlier years, and a delightful colleague always. His criticisms, if offered at all, were kindly, bearing no malice. To differ from him left no rancour, rather it cemented a liking and a

friendship. He was quiet, shy indeed, tending to efface himself and to belittle his own efforts, but to those privileged to know him he opened his heart and his mind freely, always with a leavening of quiet humour.

Here is what one of his collaborators, J. R. M. Innes, now in America, says of him :—" This is a humble tribute from a veterinarian who, as a budding pathologist, was made cordially welcome by, and ever after had a high admiration for the man that was Col. W. F. Harvey. My association with him dates back to 1927 ; in those days it was hard to find workers, medical or veterinary, who had any interest at all in veterinary pathology ; it was thus a delightful experience when I first met Col. Harvey and the late Tom Hamilton. This initial contact was the beginning of an association which lasted for the next 15 years, with frequent visits, periods of work and study, and a voluminous correspondence. As the years passed, my respect for him flourished beyond what mere words can express. I knew that Col. Harvey came to retirement in Edinburgh, having made a reputation in the Indian Medical Service which would, during a working life, have satisfied most men. It was typical of him that he succeeded, in the remainder of his days, in establishing yet another reputation in the field of morbid anatomy, particularly the pathology of tumours. I cannot forget his never-failing kindness and his sympathy for young workers like myself, and on occasion he would go to endless trouble to help by seeking out literature, information and slides.

" I cannot recall an incident when he ever said a condemnatory word of any man or his work, and I still recall a little lecture which he gave me about the avoidance of polemics in writing scientific articles.

" Someone has mentioned how he learnt to read Dutch late in life, but he was also conversant with German and French, and he astounded me by his wide knowledge of the works of German pathologists of bygone years. His industry, therefore, was yet another feature which left a deep impression.

" With the passage of years, much animal material was sent to his laboratory to be examined and reported on in his own handwriting. All who have visited that laboratory know the colossal amount of effort that was put into laboriously recording and cross indexing data in anyway relating to the pathology of tumours, and he spent just as much time over animal as over human pathological material."

The words of Marcus Aurelius, used about the philosopher Sextus, might form a fitting epitaph for Harvey :—

. . . " He let me see in himself that a man might show good-will . . . , without noise and display, and likewise possess great knowledge without vanity or ostentation."

A. M. DRENNAN
E. K. DAWSON

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Nancy Gwendolyn Shubik
1915-1948

DR NANCY GWENDOLYN SHUBIK (*née* ROGERS) died suddenly on 20th September 1948. The daughter of Mr and Mrs F. S. Rogers of Newport, Monmouthshire, she entered the Welsh National School of Medicine in 1935, taking the Conjoint Diploma in 1939 and the D.P.H. in 1941. After two years in clinical and public health work she took up the post of demonstrator in pathology at Cardiff, and it was immediately evident that she had found her right sphere. She worked with great energy and was very successful in running a clinical pathology laboratory at Llandough Hospital. Indeed her conduct of this new and responsible post contributed in no small measure to the successful collaboration of this important municipal hospital with the Welsh National School of Medicine. She was, however, more interested in the academic side and in 1944 she was appointed junior lecturer in pathology at the British Postgraduate Medical School. She remained there until 1945 when she joined the Indian Medical Service, in which also she did excellent work. On returning to England in 1947 she again took up laboratory work and was engaged in neuro-pathology in Professor Hugh Cairns's department at Oxford at the time of her death.

Throughout her career she was a most enthusiastic worker, with a great determination to seek the exact answers to the problems which she encountered in her work. She certainly under-estimated her own intelligence, while over-estimating the goodness in others, with the result that she alone was dissatisfied with her own accomplishments. She was keen on research and in collaboration with Professor E. J. King published papers in this *Journal* on the effect of olivine on the lungs of rats (1945, lvii, 488), on attempts to prevent silicosis with aluminium (1945, lvii, 281) and on a comparison of the effects of lævo-rotatory and dextro-rotatory quartz on the lungs of rats (1945, lvii, 491). She regularly attended the meetings of our Society, of which she had been a member since 1946, and many members will sadly miss her gracious presence.　　　　　JETHRO GOUGH.

Josef Flaks
1903-1949

DR FLAKS came to this country in 1946, when he was still serving in the Medical Corps of General Anders's Army. His purpose was clear. He wanted to pursue the kind of research in which he had been occupied in Poland before the war. In April 1947 he was appointed a research fellow in the Department of Experimental Pathology and

Cancer Research in the University of Leeds and he remained there until his death.

Josef Flaks was born in Plock, Poland, in 1903. He first trained as a veterinary surgeon at Warsaw University, obtaining his degree in 1927. Even as a student he began to work in the department of histology and embryology, but after graduation he was called to military service for a period of two years. In 1929 he returned to the department and embarked on the medical course, which he completed in 1935. During this period he became interested in the problem of cancer and undertook a considerable volume of experimental work along with his normal studies. He achieved the title of Doctor of Medicine of Warsaw University in 1938 by presentation of a thesis : '' L'hypothèse du virus dans les tumeurs transplantables ''.

Some of his early experiments were concerned with the influence of endocrine glands on the rate of growth of tumours. By transplanting the Jensen rat sarcoma into endocrine and other organs he observed the influence of a hormonal environment on malignant cells and established the accelerating influence of the thyroid and the inhibiting influence of the adrenals. The effect of intramuscular transplantation of the Jensen tumour into rat sucklings was next observed, and its capacity to metastasise to the regional lymph nodes in young animals was suggested as a means of obtaining suitable material for therapeutic experiments, since its growth in the lymph nodes was found to be unusually regular. Further experiments showed that the lungs of tumour-bearing rats were able to transmit tumours at a stage when there was still no sign of macroscopic metastasis. This was an interesting observation in relation to the long latent period (often many years) sometimes observed in the human subject between the excision of a primary tumour and the appearance of metastases. As the sex hormones became better understood, Flaks tested their various effects on transplanted and induced tumours and it was to this aspect of the problem that he returned when he began work in England in 1947.

In 1939 came a period of complete disruption of his research activities when Flaks was mobilised in the Polish Army. Wounded and taken prisoner by the Germans, he later escaped to Lwow, then in Russian occupation. Here he worked as a pathologist in the University until the outbreak of war with Germany in 1941. He was then sent by the Russians to Siberia as pathologist to a Russian hospital, but after the Polish-Russian agreement of 1942, he rejoined the Polish forces, this time in Russia. Later he was sent to Iraq, Egypt, Italy and finally England, taking charge of pathological laboratories in all these countries. It is sad to think that a man of such innate modesty and humility should have had to experience the perils and vicissitudes of total war as he knew it.

Flaks's interests in the post-war period were again in the field of endocrinology in relation to tumour induction. He had many

irons in the fire, but much of his time was devoted to studying the inhibiting action of testosterone on normal and malignant tissues. The investigation proved to be tedious and even inconclusive, but this was the kind of problem which Flaks liked and he had many ideas about the next step to take, which he hoped would put the matter beyond all doubt. To watch him at work was an education. Both his technical methods and his system of recording were of a very high order. A most careful observer, he missed nothing through failing to look for it. It is tragic that by his sudden death he was debarred from handing on to a pupil his enthusiasm, his extensive knowledge and his experience in research methods.

He was elected a member of our Society as recently as July 1948.

GEORGIANA M. BONSER.

BOOKS RECEIVED

Krebsmetastasen

By HANS E. WALTHER. 1948. Basel: Benno Schwabe & Co. Pp. 560: 293 text figs. Swiss fr. 60

The basis of this book is a detailed statistical analysis of the records of 3433 cancer necropsies performed at the Zurich Pathological Institute between 1927 and 1941. The writer did not perform these necropsies himself; indeed, he is not a pathologist, but a radiologist who is interested in the dissemination of tumours.

The book consists of two parts, a general and a special. The first discusses the nature of malignancy, the routes of direct spread and metastasis, the factors determining the selection of metastatic sites, and diagnostic errors caused by metastatic tumours. A main theme of this part is that metastasis by the blood stream takes place by four different routes : (1) from the lungs via the arterial blood stream, (2) from the liver to the lungs, (3) from invaded systemic veins to the lungs and (4) from invaded portal veins to the liver. In the second part of the work the tabular analyses of the Zurich necropsy records are designed to show how these modes of metastasis apply to the various groups of primary tumours. These analyses are presented in 173 tables, many of them full-page, which thus occupy a large part of the book.

As a careful, detailed and well illustrated study of the distribution of metastatic tumours recorded in the Zurich material, the book can be wholeheartedly recommended ; it also presents the well-known facts about the spread of tumours in a clear and graphic way. But pathologists will not find in it a great deal that is new, and they will question the validity of some of its analyses. In particular, no evidence is given to convince the reader of the correctness of the diagnosis in the 19 cases of " primary carcinoma " of the pleura (table 90), of the 12 cases of " Ewing-sarcoma " of bone, also called " sarcoma reticulocellulare myeloplasticum " (table 61), or of the cases of " carcino-sarcoma " described on pages 171-181. Further, the complicated histological subdivisions of the main species of tumours (for example, 15 types of carcinoma and 7 types of sarcoma of the breast in table 150) must surely be very arbitrary. In brief, the work suffers from the same difficulties as all retrospective analyses of other people's records—the difficulties of arbitrariness of nomenclature and detailed subdivision, and of doubts as to the diagnosis of unusual tumours unless clear evidence is presented of their correctness.

Of the 293 illustrations, about two-thirds are gross or microscopic photographs of the Zurich material, most of them clear and instructive. The remainder are diagrams, X-ray pictures and drawings of normal anatomy. An unnecessarily large amount of space is given in the text to accounts of the normal anatomy of each organ considered. References, of which there are many, are given in blocks at the end of sections and subsections of the text. The selection from the Continental and American literature is good but this is not always the case as regards British papers, important articles germane to the author's subject often being omitted. For example, Stewart's and Cappell's important papers on chordoma and

Dukes's work on carcinoma of the intestines are overlooked. The book is beautifully produced and misprints are infrequent, but table 79 is incomplete.

Studies in air hygiene

By R. B. BOURDILLON, O. M. LIDWELL and J. E. LOVELOCK, with 10 collaborators. 1948. Medical Research Council Special Report Series no. 262. London : H.M. Stationery Office. Pp. iv and 356 ; 104 text figs. and 33 plates. 7s. 6d.

" Studies in air hygiene " is a work which must be studied and used for reference by every epidemiologist and bacteriologist who is concerned with the spread and control of infectious disease. It is packed with useful information and first-class photographs and drawings which, as well as being valuable in themselves, are useful for teaching the principles of aerial transmission of infection.

One cannot do better than quote from the preface. ". . . this Report must be accepted as a landmark in the study of air hygiene ; for not only have various practical methods of air disinfection been assessed and compared but the one fundamental prerequisite to any satisfactory work on the subject—a reliable and easy technique for the quantitative estimation of the bacterial contamination of air—has been attained. Methods of air sampling have been developed which permit accurate determinations of the number of particles carrying viable bacteria present per unit volume of air and the minute by minute recording of their numerical fluctuation ; so that it is now possible to follow the variations in bacterial content before, during, and after the use of a disinfectant.

" The observations and experiments described in the Report fall conveniently into four main groups. In the first there are detailed accounts of the various instruments developed for sampling air, followed by an experimental and theoretical examination of the factors that may influence their efficiency. The second group is concerned with practical methods of air disinfection : chemicals, ultraviolet radiation, heat, and filtration. Attention may be directed in particular to the discovery of the value of the aliphatic α-hydroxy-carboxylic acids and to the promising experiments to achieve effective destruction of bacteria dispersed in air by the simple exposure at room temperature of a surface coated with disinfectant. Thirdly, field studies were organised to measure levels of bacterial contamination of air in operating theatres, dwelling houses, factories, air-raid shelters, and in warships under active service conditions. Lastly a few animal experiments on the transmission of airborne infections are described. The Report ends with a critical evaluation of the different methods of air disinfection that are now available."

Experimental air-borne infection

By THEODOR ROSEBURY. 1947. Baltimore : The Williams and Wilkins Co. (London agents Baillière, Tindall and Cox). Pp. xi and 222 ; 45 text figs. 22s.

This monograph, the first of a new series to be published by the Society of American Bacteriologists, presents the results of a co-operative war-time investigation at Camp Detrick, Maryland. Equipment was devised which permits infection of animals with air-borne clouds of highly infective agents while ensuring the safety of the operators. Section i of the report describes the history and scope of the project and briefly reviews previous work. Section ii gives details of the furnishing and working of a laboratory

in which infective clouds could be generated and animals introduced into the cloud, maintained, removed, autopsied and disposed of, all in a completely closed system. In section iii there is a survey of types of atomiser, and experimental results are given which support the choice of an all-glass, direct-spray instrument for this investigation. Section iv presents basic data such as the methods of sampling airborne clouds into fluid media and theoretical considerations of cloud development and particle size. The selection of media for cloud-sampling and the influence of humidity, temperature and other conditions on the stability of clouds during atomisation are discussed in the last section, which also describes experimental infections. *Pasteurella tularensis* and *Malliomyces pseudomallei* (Whitmore's bacillus) produced a higher rate of infection and death than other bacteria used. Experiments were also carried out with the viruses of psittacosis and meningo-pneumonitis. Some of the agents were destroyed in varying degrees by atomisation, though the reasons for this are not clear. The purpose of the investigation was to elicit reproducible quantitative data on infection by the inhalation route and, in the main, this aim was achieved. In some respects the book is complementary to the recently published Medical Research Council's "Studies in air hygiene" (*vide supra*) but it will chiefly interest those concerned with experimental epidemiology.

Fourth International Congress for Microbiology, 1947 : Report of Proceedings

1949. Copenhagen : Rosenkilde and Bagger. Pp. 649.

The work of the Congress is reported in nine sections, namely general microbiology, medical and veterinary bacteriology, viruses and viral diseases, serology and immunology, variation and mutation in micro-organisms, plant pathology and mycology, soil and water microbiology, dairy and food microbiology, and industrial microbiology. Besides the summarised account of the papers given to the Congress there are full texts of the four communications which were given at the general sessions : "Heterotrophic assimilation of carbon dioxide" by C. H. Werkman, "Some implications and limitations of plant viruses" by F. C. Bawden, "Antibiotics and life" by S. A. Waksman, and "Yeast in modern genetics" by O. Winge. This volume is indispensable to all serious workers in bacteriology.

Applied medical bacteriology

By MAX S. MARSHALL, with 4 collaborators. 1947. London : Henry Kimpton. Pp. 340 ; 10 text figs. 22s. 6d.

This is a new volume on clinical bacteriology and is much more satisfactory than some that have been published in this field. The authors have kept well to the lines they laid down for themselves. They set out to save "applied medical bacteriology" from one of two undesirable fates ; they wished to avoid its being unduly compressed, as in the usual text-book which attempts to cover the whole field of clinical pathology, including hæmatology, bacteriology, histology and biochemistry ; they also wished to ensure that they would not produce simply another text-book of bacteriology with all the laboratory jargon which alienates clinicians. Throughout they have been successful in their efforts to produce a book which gives real guidance to those in charge of patients about the help the laboratory can give them. The detailed instructions about taking

and submitting samples are excellent. The methods advised for use in the laboratory are sound and reliable and the book may safely be recommended for those who have to work on their own.

Pathology and surgery of thyroid disease

By JOSEPH L. DeCOURCY and CORNELIUS B. DeCOURCY. 1949. Springfield, Ill. : Charles C. Thomas. Pp. xviii and 476 ; 120 text figs. (4 in colour) and frontispiece (in colour). 50s.

This book sets out the experience of many years' work in a well-known thyroid clinic in Cincinnati : it gives a wide survey of the literature, with a well-balanced and unbiased judgment on controversial issues. Naturally there is an inclination towards surgical considerations, but there are also helpful discussions on the embryology, histology and pathology of the thyroid. Physiological and general therapeutic aspects are adequately dealt with, including the use of anti-thyroid drugs and radioactive isotopes of iodine. The detailed instruction on the surgical aspects of treatment include both pre- and post-operative care, and there is an interesting chapter on thyroiditis, of which the authors have had considerable experience.

The book is beautifully produced, with numerous diagrams and plates, some of them in colour, and the print is clear and free from typographical errors. A comprehensive bibliography is provided for each chapter. The book can be recommended as an important contribution of particular value to surgeons interested in thyroid disease.

Bronchiogenic carcinoma and adenoma

By B. M. FRIED. 1948. London : Baillière, Tindall and Cox. Pp. xiv and 306 ; 184 text figs. 33s.

The diagnosis of malignant disease in the thorax can no longer be regarded simply as a feat of diagnostic gymnastics, for to-day the removal of a lung affected by cancer may be a feasible and relatively safe surgical procedure. The object of this book is to assist in the identification of the disease in its incipient stages, a tardy diagnosis being regarded by the author as the chief obstacle to successful treatment. Thus approximately one-half is devoted to pathological aspects of the subject—incidence, morbid anatomy, histogenesis and aetiology—and the remainder to clinical manifestations, laboratory methods of diagnosis and treatment.

The first chapter is concerned with the much-discussed question as to whether there has been a real increase in the incidence of intra-thoracic cancer during this century and especially since the first world war. It would be impossible to marshall all the evidence in a book of this size, and the bias is naturally towards American statistics. The author states clearly, however, that in his view the observed increase is more apparent than real.

In discussing histogenesis, all lung cancers are relegated to an origin from bronchial epithelium and, indeed, in the author's view, from the basal layer. Origin from cells lining the alveoli and from mucous glands is not admitted. The microscopical classification adopted is unusual, all types except adenocarcinomas (simple, papillary and mucocellular) and round cell tumours being classed as squamous-cell cancers. The latter thus include the oat-cell tumours and constitute 73 per cent. of all cases. As oat-cell areas can always be found in the kind of round-cell tumour illustrated (fig. 22), the incidence of squamous cancer when this classification is used would be even higher. Adenocarcinoma is noted as occurring five times more frequently in women than in men, and as displaying more

malignant tendencies than other varieties, as shown by the clinical course and the distribution of metastases. The photomicrographs are not impressive.

The chapter on ætiology touches on every aspect of cancer research and leaves the reader little wiser as to the cause of lung cancer. The lack of ætiological association between lung cancer and tuberculosis, tarring of the roads, tobacco, silicosis, influenza, viruses and trauma and its probable association with chromates, asbestos and radon emanations (the latter in the Schneeberg miners) are accepted.

There is an interesting chapter on metastasis, attention being drawn to the high incidence of bone metastases from lung cancer in the author's series.

Following a good description of the clinical manifestations of the disease, laboratory diagnosis by means of X-rays, bronchoscopy, aspiration biopsy, and the microscopic examination of the pleural exudate, sputum and bronchial secretions is discussed and there is a short section on treatment.

A chapter is reserved for bronchial adenoma, origin from both bronchial mucous glands and from the basal cells of the bronchial epithelium being accepted. This tumour is differentiated from bronchial carcinoma by its symptoms and physical signs, by its appearance on bronchoscopy and by its histological structure. The accompanying illustrations are of a high order. Mediastinal tumours are also briefly considered.

To each chapter is appended an extensive bibliography, in which, regrettably, there are many errors.

This book falls between two stools—it is not sufficiently detailed for the pathologist or those experienced in cancer research and it is hardly likely to satisfy the chest physician or thoracic surgeon. One of its chief values lies in the unequivocal manner in which the author expresses his own opinions, formed from vast experience, on controversial aspects of the subject.

Pathology of the nervous system

By J. HENRY BIGGART. 2nd ed., 1949. Edinburgh : E. & S. Livingstone. Pp. xii and 352 ; 233 text figs. and 10 plates in colour. 21s.

In the second edition of this excellent little manual the subject-matter has been very skilfully brought up to date without much expansion of the text ; the main additions are a number of excellent half-tone illustrations and 10 photomicrographs in colour. The author, in his revision, has profited from criticisms of earlier reviewers and this new edition should prove even more widely acceptable than the first. Nevertheless it is doubtful whether many will agree that cerebral hæmorrhage is much more uncommon than thrombosis (p. 64) or that brain abscesses usually rupture into the ventricles (p. 90) ; and if there were in fact no stroma in medullo-blastomas (p. 290), would not all the tumour tissue be washed away by the cerebro-spinal fluid ? The important question of the routes followed by pyogenic infections invading the intracranial tissues is treated somewhat cursorily and the diagram from Turner and Reynolds, reprinted on too small a scale on p. 88, is a poor substitute for an adequate verbal summary of their admirable work. The book is written in a simple, clear and easy style that earns one's gratitude, but it sometimes conveys an impression of undue dogmatism which could be mitigated by a more generous citation of authorities, with the dates of their contributions. Another minor defect is the occasional use of words without regard to their accepted meaning; " anoxæmia",

for example, is repeatedly employed to denote the tissue anoxia which results from vascular obstruction. Nevertheless this is a book that we can read with profit and recommend to our students without reserve.

Tumors of bone

By CHARLES F. GESCHICKTER and MURRAY M. COPELAND. 3rd ed., 1949. Philadelphia, London, Montreal : J. B. Lippincott Co. Pp. xviii and 810; 642 text figs. $17.50.

This large book has the merits and demerits of previous editions. Its merits are that it is based on a large and comprehensive collection of material, that this is profusely illustrated in radiographs and gross and microscopical photographs, and that it is beautifully produced. But as a work on pathology it is disappointing ; many of the conclusions are uncritical and insecure, and there are many omissions and contradictions. Thus of osteochondromas it is stated that " these tumors are an exaggeration of normal bony protuberance intended for the anchoring of an important tendon " (the wording is the authors') ; of the origin of multiple exostoses, " tags of perichondrium in the tendon ends proliferate to form cartilaginous and bony outgrowths " ; " most of the chondromas and chondromyxomas represent histogenetically supernumerary joint cartilages " ; " usually, the single bone cyst is . . . an arrested giant-cell tumor " ; and of metastatic carcinomas in bone marrow, " in the majority of them Bence-Jones bodies have been demonstrated ". The authors persist in their belief that osteoclastoma is an epiphysial tumour and that giant-cell tumours of tendon sheaths arise in sesamoid bones. They say that " three cases of adamantinoma of the lower tibia have been reported " (the actual number is nearer 20), and they deny the existence of solitary plasmocytomà of bone, accordingly omitting all references to the many papers on this subject. Of the contradictions in different parts of the text, those concerning neuroblastoma and Ewing's tumour are the worst. Thus on p. 521 we are told that neuroblastoma occurs " in children under the age of five years", and on p. 728 that of 12 cases of this disease in which metastases were present in the spine " the average age of onset was 33 years, the extremes of age being 15 and 66 years", and that the authors are aware of many other recorded cases in adults. Then, on p. 424, " the age distribution (of Ewing's tumour) is against metastatic neuroblastoma " ; yet, on the previous page, this tumour occurs in " 95 per cent. of cases in persons under 25 years of age". Incidentally, fig. 294, " showing the characteristic cell of Ewing's sarcoma", is a particularly bad one and shows nothing characteristic of anything.

For a special work of this kind there are some serious omissions. It contains nothing about the comparative pathology or experimental production of bone tumours, and next to nothing about their causation in man. The section on endocrine disturbances is so slight and incomplete that it had better have been omitted. In the 65 pages on metastatic tumours in bone, neither Willis's nor Walther's books on metastasis are mentioned ; and, although the reference lists are long, many essential papers on particular subjects, especially British, are missing. The magnification of none of the photomicrographs is stated, and there is much needless repetition of figures. Thus fig. 431, which with its verbose legend occupies three-quarters of a page, is the *fourth* appearance of fig. 7, and figs. 73 and 74A are not only repetitions of 11G but actually appear on facing pages. The length of many of the tables is out of proportion to the amount of useful information they contain, and some of them, *e.g.* 69, have very little meaning. The index is unreliable ; *e.g.* neuro-

blastoma is indexed for one page only, and sympathicoblastoma not at all, although both occur frequently in the text ; and Ollier's disease appears in the index but not in the text. Misprints are unduly numerous.

Diagnostic bacteriology

By ISABELLE GILBERT SCHAUB and M. KATHLEEN FOLEY. 3rd ed., 1947. London : Henry Kimpton. Pp. 532. 22s. 6d.

This edition of " Methods for diagnostic bacteriology " has a new title and now includes theoretical discussion of the techniques described. As in previous editions emphasis is placed on hospital bacteriology and explicit instructions are given for isolating pathogens from clinical and autopsy material. The chapter on the recognition of bacteria by their colonial characters is useful, but the presentation is needlessly diffuse and relevant information on any one organism must be sought in different parts of the book. In the section dealing with serological diagnosis there is unnecessary repetition in describing the typing of pneumococci isolated from different sources and, while the serological types of Friedländer's bacillus are included, the value of the Vi- test for detecting typhoid carriers is not mentioned. In the final section, formulæ for various bacteriological media and stains are given, together with descriptions of such tests as the oxidase reaction for the gonococcus and the ferric chloride method for detecting hydrolysis of sodium hippurate. This book is intended for students and trainee technicians but it seems unlikely that it will be widely used in this country.

Hygiene

By J. R. CURRIE and A. G. MEARNS. 3rd ed., 1948. Edinburgh : E. & S. Livingstone, Ltd. Pp. xx and 724 ; 212 text figs. (21 in colour) and 4 colour plates. 35s.

" Hygiene " by Currie and Mearns is yet another textbook in public health which has appeared recently as a new edition largely as a result of the far-reaching effects of the National Health Service Act. A serious attempt has been made to rewrite the book, but a number of errors occur which show that the authors have not completely avoided the traps that the new act has created. For instance the Blind Persons Act, 1920, sections 2, 3 and 4 of the Blind Persons Act, 1938, and the prevention of blindness in the Public Health Act, 1936, are quoted although all have been repealed by the National Assistance Act.

Some sections of the book are very good, particularly those on mental health and statistics, but some of the sections dealing with preventive medicine do not seem completely up to date. Under the subject of anthrax, for example, it is stated (p. 101) that " The most effective treatment is the intravenous injection of Sclavo's anti-anthrax serum".

The section on industrial hygiene is quite good, although the Workmen's Compensation Act, quoted extensively, has now been repealed and it seems a pity, with the publishing date 1948, that the authors did not concentrate on the future procedure under the National Insurance (Industrial Injuries) Act, 1946, rather than on what was obviously destined to become out of date within a short time.

Under the subject of silicosis it is stated that " the disease is not a normal hazard of coal mining ". As an unqualified statement this gives a very incorrect picture of the real situation.

A number of coloured illustrations are provided, but the colours of some of them are very unnatural. The delicate pictures of *Amanita phalloides*

and *Amanita muscaria* are very attractive, but one wonders whether they are really necessary.

The book is well written from the Scottish point of view, but the handling of English public health law is not so satisfactory and the different procedures in the two countries, particularly in regard to maternity services, are not made sufficiently clear for the average student. The kilt is strongly advocated as the ideal dress for boys and the reviewer feels that the book will probably be greatest value to (and most appreciated by) those who subscribe to this view.

Sternal puncture

By A. PINEY and J. L. HAMILTON-PATERSON. 4th ed., 1949. London : William Heinemann, Medical Books, Ltd. Pp. xi and 89 ; 1 text fig. and 27 figs. (22 in colour) on 18 plates. 15s.

The addition of 3 figures, 2 colour plates and 10 pages of text has added as little to the stature of this book as to its bulk. The two new colour plates depicting various types of plasma cells in myeloma and of megaloblasts in pernicious anæmia are tinctorially better than the older plates, but their usefulness is marred by the fact that the numbers attached to the various cells or cell groups do not correspond to the numbers in the descriptive legends. This, alas ! is not the only evidence of carelessness in preparing the book. For the explanation of fig. IV, for example, the reader is still referred to p. 74, an instruction which was correct in the last edition but which should have been amended to p. 83 in this. The chapter on " Neoplastic and allied conditions of the bone-marrow " has been redrafted and expanded, and other additions to the text include such matters as the subdivision of types of megakaryocytes, the effect of urethane on the myelogram in leukæmia and the crises of acholuric jaundice. But these additions savour strongly of a few chance dips into the bran-tub of the recent literature. The book still lacks authority and seems to be better suited to the needs of the physician who requires only a nodding acquaintance with the subject than to the needs of the pathologist, who soon encounters problems that cannot be resolved by reference to this volume.

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HEPATIC CHANGES IN YOUNG PIGS REARED IN A COLD AND DAMP ENVIRONMENT

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(PLATES LXXVII-LXXIX)

THE hepatic changes described in this paper are essentially those which McGowan (1924) attributed to iron-deficiency anæmia in suckling pigs reared without access to their natural sources of iron in soil and grass. It is now well established that the administration of iron in suitable doses prevents the low hæmoglobin levels—20-30 per cent. Haldane—seen during the first 4 weeks of life in suckling pigs (Venn et al., 1947). But in spite of iron administration it remained difficult to rear healthy pigs indoors in the north-east of Scotland, especially during the colder months of the year from October to March. Pigs reared in small wooden ark huts on pasture, however, attained good weights and showed excellent health when weaned at 8 weeks of age. The difference between the " outdoor " and " indoor " pigs was investigated experimentally by Howie, Biggar, Thomson and Cook (1949), who showed that the complete sequence of events attributed by McGowan to iron-deficiency anæmia of suckling pigs could not be prevented by giving large doses of iron by mouth, but could be prevented by giving the young animals, in addition to iron, a warm, dry and comfortable environment, instead of attempting to rear them in a damp, draughty and cold byre largely built of concrete, whose floor was not insulated to prevent loss of heat. For reasons fully stated in their paper, Howie et al. regarded the warmth and comfort of the wooden ark huts rather than the iron or other nutrients in the soil and grass as the reason for the superior health of pigs reared outdoors. Independently, Inglis and Robertson (1949) also emphasised that many of the communal pig-houses built in this

country in the past 30 years or so had a bad effect on the health and productivity of pigs because they were too cold during the winter.

The description given by Howie *et al.* of the pathological changes they found was confined to a brief outline, sufficient to indicate the general nature of the lesions in the pigs which died. McGowan and Howie *et al.* alike emphasised the liver changes as a main pathological feature of the disease they studied, and their descriptions agreed in so many respects that it would seem they were dealing with the same condition though attributing it to different causes. The work described in the present paper was undertaken therefore to furnish a detailed description of the pathological changes in the liver of young pigs reared in a cold and damp environment, to compare these lesions with those described by McGowan, and to investigate whether a close examination of the morbid anatomy and histology of this still unnamed disease of pigs would throw light on its pathogenesis. This seemed to be necessary since we failed to find an account of the pathological effects in man or animals of exposure to excessive cooling over periods of from 3 to 15 weeks, although there were descriptions of acute experiments involving brief exposure to all degrees of cold. The need for information on the effects of climatic stress on animal health was emphasised in an admirable review by Lee and Phillips (1948).

EXPERIMENTAL PROCEDURES

Nine litters used in 3 experiments yielded 67 piglets alive three days after birth, *i.e.* after the period during which many piglets die from being overlaid by the sow. The experiments were designed to compare the effect of a comfortable with that of a cold and damp environment on the growth and health of suckling pigs from birth to the age of 8 weeks, when they were weaned. Pigs which survived beyond 8 weeks remained under observation, but in an environment which was not regulated, being warm and comfortable for some litters or half-litters and cold for the others.

The litters were born during the cold months from October 1948 to March 1949. Litters were allocated to one of three groups according to the type of housing as detailed by Howie *et al.* For brevity, these may be described as follows. **Group 1,** Open pen in a cold, draughty and often damp byre, with a non-insulated floor and only a limited amount of straw for bedding. **Group 2.** Warm shelter provided by a wooden ark hut or an electric hoover. The pigs had an indoor run to a pen within the same byre as group 1. **Group 3.** Wooden ark hut providing shelter as in group 2, but the pigs had an outdoor concrete run adjacent to the byre inside which groups 1 and 2 were housed. After being reared to weaning in good health in the conserved warmth of wooden arks or an electric hoover, the pigs were still unable to tolerate the cold conditions of the open pen in the draughty byre. Accordingly, 4 pigs which were transferred to this environment after having been raised to weaning under an electric hoover were regarded as belonging to group 1. All the piglets received 1·0 g. of reduced iron by mouth on the 3rd, 10th, and 17th days of life. This, among pigs which are comfortably housed, is enough to prevent a fall of hæmoglobin to anæmic levels, which, for pigs of 3-4 weeks of age, may be regarded as levels below 40 per cent. Haldane. Table I shows the distribution of pigs, the hæmoglobin levels (per cent. Haldane), and the incidence of deaths among the three groups. It is seen that the Hb. levels were not significantly different at 3 weeks

of age, the period when hæmoglobin reaches its lowest level among pigs reared without access to additional iron. After 3 weeks of age, healthy pigs begin to eat solid food in increasing amount and this quickly leads to a rise in hæmoglobin in animals which until then have depended entirely on the relatively small amount of iron available from sows' milk. The animals were observed daily

TABLE I

Distribution of piglets and incidence of deaths between 3 days and 15 weeks of age among three groups whose environments differed in cooling power

Experimental group	Type of house and run	Nature of environment	Group mean hæmoglobin per cent. (Haldane) at		No. in group at 3 days of age	No. * dead from 3 days to 15 weeks
			3 weeks	8 weeks		
1	Open pen in cold, damp, draughty byre with non-insulated concrete floor	Cold, damp, and draughty at all times	64·5 (22)	39·4 (14)	30	16
2	Wooden ark hut; indoor run on concrete as in group 1	House warm and dry; run cold, damp and draughty	67·6 (16)	66·8 (11)	21	5
3	Wooden ark hut; outdoor run on concrete	House warm and dry; conditions in run varied with weather	67·4 (16)	78·8 (16)	16	0

* The first death was observed on the nineteenth day of life (see table II)

All piglets received by mouth 1·0 g. of reduced iron on the 3rd, 10th and 17th days of life. Hæmoglobin estimations were not made on all piglets, and the figures in parenthesis in columns 4 and 5 show the number of animals used in determining the group means

and their Hb. levels were ascertained at 3, 6 and 8 weeks of age with a N.P.L.-calibrated Haldane hæmoglobinometer, on which a reading of 100 per cent. was equal to 14·8 g. of Hb. per 100 ml. of blood. Normal adult pigs in good health with access to soil and grass had hæmoglobin values of 80 per cent. on the same instrument. The rate of growth of the animals was checked by weekly weighing.

RESULTS

Incidence of deaths

It will be seen from table I that in group 1, the coldest group, 16 of 30 pigs died. In group 2, which was more warmly housed, only 5 of 21 pigs died. In group 3, where the housing was warm and the animals had access to the open air but not to soil or grass, there were no deaths among 16 pigs. The 21 pigs that died were examined *post mortem*, attention being devoted mainly to the liver, which showed the most obvious lesions. Summarised data for each pig that died are given in table II.

Clinical features of sick animals

After being apparently in good health up to 3 weeks of age, litters of pigs housed in cold and damp conditions began to show important differences from those housed in warmth and comfort. The most

TABLE II

Liver pathology of 21 pigs dying between 19 days and 19 weeks of age. The animals are arranged in order of age at death

Pig no.	Experimental group (see table I)	Age at death (days)	Weight of — pig at death (g)	Weight of — liver (g)	Weight of — g/100 g body wt	Appearances of liver — Macroscopic	Appearances of liver — Microscopic	Ascitic fluid (c.c.)	Other post-mortem findings and notes
1	2	19	2800	120	4·3	No abnormality observed	Some areas normal, in others sinusoids congested or dilated and empty of cells (figs. 4 and 5) Red blood cells in tissue spaces Liver cells vacuolated and fatty	See next column	Extensive hæmorrhage into pericardium, pleura and peritoneum. Organising fibrinous pericarditis. This pig suffered severe crushing in the first 3 days of life Femoral marrow not hyperplastic
2	1	27	7050	495	7·0	Surface deep purple. Numerous yellow spots of pin-head size, with dark centres Lobules enlarged	Graded changes from normal to lobules with fragmented columns of liver cells and lobules with central and mid-zonal necrosis, around which the liver cells show fatty change and nuclear pyknosis Central and mid zones of many lobules packed with r.b.c. and only a peripheral rim of liver cells surviving In other lobules, central and mid zones empty. Many sinusoids in central zone dilated	100	...
3	1	33	8175	570	7·0	Lobules enlarged with dark centres and yellow periphery: typical nutmeg liver	As 2, plus enlarged Kupffer cells and fibroblastic perilobular tissue	150	...
4	1	37	8700	720	8·3	Nutmeg liver	As 3	350	...
5	2	40	10,200	667	6·5	,,	,,	350	...
6	2	40	10,750	590	5·5	,,	,,	0	Organised peritoneal exudate Femoral marrow not hyperplastic
7	1	40	5750	387	6·7	,,	As 2	230	...
8	1	40	6100	490	8·0	Nutmeg liver, some yellow areas measuring 2-3 mm	As 3. Yellow areas show fatty change at periphery of lobule. Periportal areas show a few found cells	100	...
9	1	42	10,550	850	8·1	Nutmeg liver	As 3, but some lobules show only fatty change without necrosis around central vein	750	...
10	1	43	10,000	650	6·5	Nutmeg liver ; caudate lobe yellow	As 2	200	Spastic paralysis of hind legs on day of death. Femoral marrow not hyperplastic

11	2	17	12,050	905	7·6	Typical nutmeg liver. Yellow areas up to 2 cm. in diameter. Fibrous tissue increased	Liver cells at periphery of lobules vacuolated. Central and mid-zonal necrosis with little fatty change. Kupffer cells enlarged. Sinusoids dilated and walls thickened; mostly packed with red blood cells. Areas of well-marked central, mid-zonal and periportal fibrosis (fig. 10), with vascularisation in some areas	320	Pleural fluid 120 c.c.
12	2	18	10,150	805	8·3	Nutmeg liver	As 2, plus very œdematous perilobular fibrous tissue. Some lobules also show central œdema and fibrosis	275	Organised peritoneal exudate
13	1	65	1800	280	5·8	Numerous pale areas up to 0.5 cm. in diameter	As 3, but perilobular fibrosis more marked	80	,,
14	1	71	1550	235	5·2	Liver lobules small. Nutmeg liver. Capsule thickened	Pale areas show early abscess formation	0	,,
15	1	78	5000	310	5·8	Pale and soft; alternate areas of light grey and yellow	Liver cells atrophic, with small nuclei; cytoplasm well stained but scanty. Perilobular and intralobular fibrosis. Other changes as in 2	0	Hæmoglobin levels (per cent Haldane) at 3, 6 and 4 weeks = 54, 22 and 27. Organised peritoneal exudate
16	1	81	20,200	1600	8·2	Nutmeg liver with yellow tint at lower edge	As 3, but many lobules show extensive infiltration of their necrotic areas with lymphocytes, eosinophils and large mononuclears	200	,,
17	1	82	10,050	1105	7·4	As 18	As 2 with more marked congestion and less fatty change (fig. 6)	50	,,
18	1	80	10,125	1050	10·2	Nutmeg liver, much enlarged (fig. 2). Lobules also much enlarged	As 10	80	,,
19	1	90	20,000	1705	8·0	Nutmeg liver	Dark congested areas as in 2 but congestion more marked. Ischæmic areas with large pale vacuolated liver cells and only scanty normal cells (fig. 7). Slight fatty change and fibrosis	0	Organised peritoneal exudate
20	1	99	9050	295	1·9	Atrophic liver, small lobules, marked fibrosis	As 18	0	,,
21	1	101	10,550	780	7·4	Nutmeg liver with small area of fibrosis (fig. 3). Lobules enlarged	Intra- and perilobular fibrosis ++. Recent necrosis of central zones of lobules. Veins dilated; sinusoids congested. Vein walls hyaline and fibrotic	175	Pleural fluid 350 c.c.

Normal pigs (killed)

1		13	11,025	125	3·6	See fig. 1	See figs. 8 and 9	0	
2		18	13,300	110	3·7	As in fig. 1	As in figs. 8 and 9	0	
3		19	10,600	390	3·7	,,	,,		

noticeable and consistent feature of the pigs in group 1 was their unwillingness to eat the solid food which was offered from about the 17th day of life. The pigs in group 2 had better and those in group 3 still better appetites. The pigs in groups 2 and 3 retained the playfulness characteristic of young pigs in good condition, whereas those of group 1 lost this quality. At times they were listless and apathetic. When, as was usual, they huddled together in clusters for warmth, individual pigs displayed a fair amount of energy in seeking a favourable position. It was often observed that a cluster was disturbed by the members which lay with their abdomens pressed to the floor. These animals, if they were strong enough, forced their way out, ran around for a little, and then tried to climb on the backs of those remaining in the heap, which was never long at rest. On the other hand the animals in groups 2 and 3, when at rest in their hut, lay snugly and quietly on the floor, fairly close together and to the sow, but not pressed into a tight cluster. After 3 weeks, the pigs of group 1 showed great variability in growth rate. Some continued to gain weight, but in an ever-increasing number growth became retarded 'or ceased altogether. One of the most regular and most noticeable features of the pigs in group 1 was the development of humping of the back and abdominal distension, shown by radiography and at post-mortem examination to be due to the presence of ascitic fluid in amounts ranging from 150 to 700 c.c. Of the 21 pigs examined *post mortem*, 13 had ascites, 3 ascites and organising peritoneal exudate, and 5 organising exudate alone. The hair of the animals in group 1 grew long, rough and curly and was lacking in lustre, the tips of the ears were often blue and many of the animals had intermittent diarrhœa, but specific pathogens were not isolated on repeated examination of the stools.

Many of the deaths took place during the night, but in both the present and the previous experiments, two modes of death were observed. In the first the animals suddenly developed spasmodic breathing and cardiac overaction and often died suddenly after exertion. This sequence is known among pig farmers as " thumps." In the second the listlessness observed in many pigs became increasingly marked and the skin was cold and clammy. Respiration became shallow and consciousness was gradually lost. Death followed in from 12 to 36 hours from the onset of the final symptoms. The picture closely resembled that described as oligæmic shock.

Post-mortem findings

In all cases the serous cavities were inspected and the abdominal and thoracic viscera examined by the naked eye and histologically. In 3 animals the shaft of the femur was divided to explore the bone marrow. In a few animals the thyroid and adrenals were examined. In some animals the heart was moderately dilated. particularly the

FIG. 1.—Liver of normal pig no. 1 (table II), aged 43 days   Weight of liver 425 g ,
= 3 6 g per 100 g body weight   × ca. 0 5.
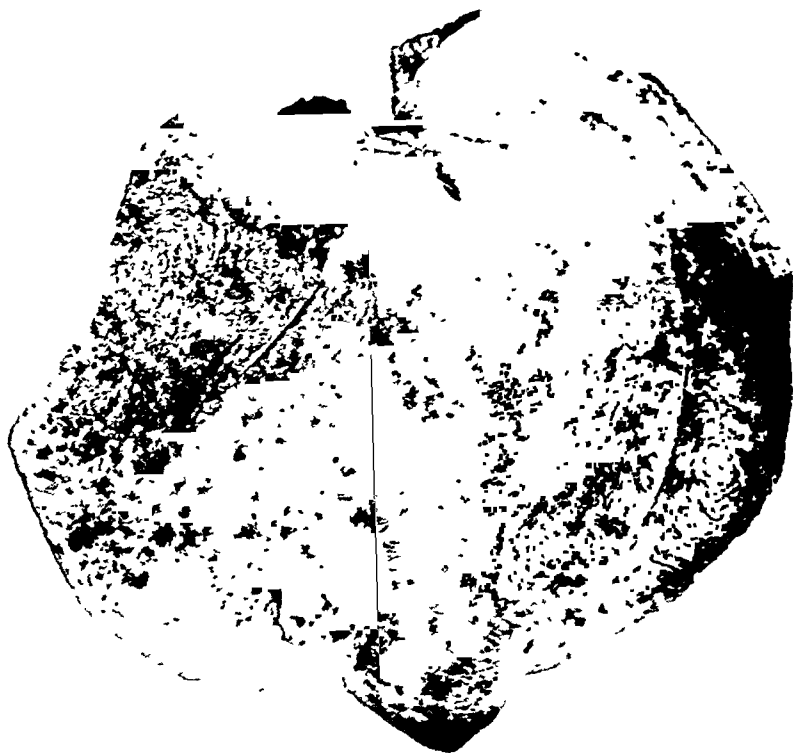
FIG. 2 —Enlarged and congested liver of pig no. 18 from cold environment (group 1),
showing areas of ischæmia and congestion. Weight of liver 1650 g., = 10 2 g.
per 100 g. body weight. Age at death 86 days. × ca. 0 5.

right side, but this was not constant. Occasionally there was a moderate effusion in the pleural and pericardial cavities. Only two consistent pathological features were disclosed: (1) ascitic fluid or peritoneal adhesions and (2) gross changes in the liver. Accordingly attention was directed to a study of the liver changes which form the subject of this paper.

Changes in the liver

Naked-eye appearances. As shown in table II the livers were usually enlarged, the range being from 4·3 to 10·2 g. per 100 g. body-weight, compared with 3·3, 3·6 and 3·7 g. per 100 g. in 3 normal pigs killed at 49, 43 and 49 days of age (figs. 1 and 2). The external surface showed all degrees of vascular congestion and was usually dark purple in colour, but pale ischæmic or fatty areas were irregularly distributed throughout the liver and were most often observed near the inferior edge of the organ (fig. 2). Enlargement of the lobules was visible on both the external and the cut surface, the latter presented the typical appearance of nutmeg liver (fig. 3). If the animal survived, the soft friable liver found in pigs dying early in the disease was gradually replaced by fibrous tissue. In one animal (pig 15), which died at 78 days, the surface of the liver showed alternate areas of light grey and yellow and the organ contained very little blood.

Histological appearances. In each animal the liver shows essentially the same lesion, but apparent diversity arises from the different proportions of its component elements, even within adjacent areas of the same liver. The components of the lesion are :—vascular congestion (fig. 6), the sinusoids being greatly distended with red blood cells ; escape of red blood cells into the tissue spaces, the sinusoids being dilated but empty of red blood cells (figs. 4 and 5) ; ischæmic areas with sinusoids closed and liver cells swollen (fig. 7) ; fatty change in liver cells ; vacuolation (fig. 5) ; loss of staining affinity (fig. 7) ; fragmentation of columns of liver cells and necrosis of individual cells ; and fibrosis (fig. 10).

DISCUSSION

The clinical features and lesions described in this paper are not those which might be expected in uncomplicated iron-deficiency anæmia. The condition was neither prevented nor cured by administration of iron but was prevented by substituting a warm and comfortable environment for one that was cold and damp ; the bone marrow, examined in 3 pigs, was not found to be hyperplastic and the liver pathology was not that associated with anæmia.

The lesions in our pigs are in all essentials identical with those described by McGowan. For example, all the abnormal naked-eye appearances of the liver shown in McGowan's composite fig. 2 were observed in our series and our fig. 3 has the essential features of McGowan's fig. 2c, d and e. McGowan's fig. 3, showing intense

congestion and hæmorrhage, especially of the central part of the lobule, corresponds to an appearance which was very common in our series, and our fig. 6 shows that the liver cells of some lobules were almost entirely replaced by blood. The dilated sinusoids in our figs. 4 and 5 are similar to those shown in McGowan's fig. 3, although he did not include them in his description. Fatty change and the later development of cirrhosis are common to both series.

Can the lesions described by McGowan and ourselves be explained as the direct or indirect outcome of exposing young pigs to excessive cooling ? Air temperatures did not differ greatly between the wooden ark hut and the open byre, but the subjective sensation was one of warmth in the wooden ark hut and of cold in the open byre. The different sensations probably arise from the good insulation provided by the ark hut with its small cubic capacity and its wooden walls and floor with an air space below the floor. By contrast the byre with its large cubic capacity and high rate of air change was draughty, the uninsulated floors and often damp bedding and walls favoured heat loss by conductivity and there was a large area of concrete floor and wall likely to cause heat loss by radiation. The cooling power of an environment can be measured by methods described by Bedford (1946) and Kelly, Heitman and Morris (1948) but we had not the facilities to apply them during our present experiments. There was no doubt, however, about the subjective sensation of warmth in the one environment and of cold in the other.

The young pig, unlike the sheep with its wool or the older pig with its fully developed layer of fat, is poorly protected against cold, and we were able to prevent the development of this disease only when we added a wooden ark hut to the environment. Continued cold will constrict the peripheral vessels, driving blood into the systemic circulation and thus raising the venous pressure. The naked-eye and microscopic appearances of the liver which we describe are essentially those of a vascular disturbance such as is associated in the human subject with increased venous pressure (Himsworth, 1947). Bolton and Barnard (1931) produced similar lesions in the liver of cats by applying a constricting band to the inferior vena cava in the chest. The idea that a vascular disturbance within the liver itself might partly account for the lesions we observed was strengthened by the absence from most of the animals of signs of chronic venous congestion in organs other than the liver. In discussing the pathogenesis of central zone hepatic necrosis Maegraith *et al.* (1947) emphasised the importance of obstruction to the blood-flow arising from changes in the hepatic vessels themselves and not necessarily from cardiac failure. They considered (p. 782) that " an active obstruction due to constriction of some elements of the hepatic venous vessels " was an important factor in the production of the lesion.

The hepatic venous tree of the pig contains a considerable amount of muscle (fig. 11) and it is not therefore difficult to imagine that

FIG 3 —Cut surface of liver of pig no 21 from cold environment (group 1), showing nutmeg appearance, with enlargement of lobules except in the fibrotic area to the lower left of the specimen   Organised exudate on surface. Age at death 101 days   Very slightly enlarged

FIG 4 —Dilated liver sinusoids around a sub lobular vein—an early stage of the lesion Hæmatoxylin and eosin.   ×70
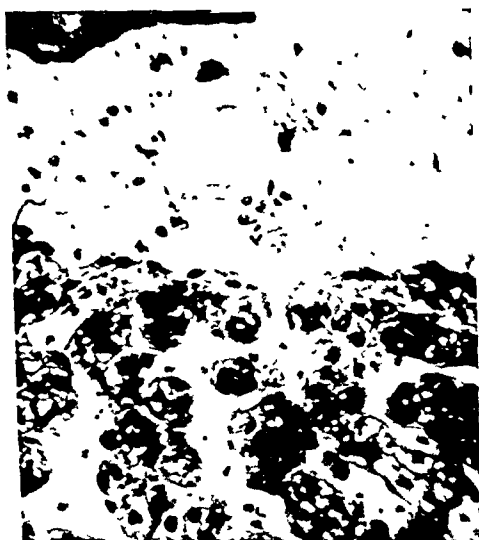
FIG 5 —High power view of part of fig. 4, showing dilated sinusoid (below) opening into a sub lobular vein (above)   Vacuolation of liver cells   Hæmatoxylin and eosin. ×340

PLATE LXXIX

FIG. 6.—Liver of pig no. 16 (group 1), showing intense congestion of some lobules and ischæmia of others. The lobules are approximately twice the size of those of the normal pig (figs. 8 and 9). Masson's trichrome stain. ×27.

FIG. 7.—Pig no. 18 (group 1). Ischæmic area with large pale liver cells, among which a few darkly stained normal cells are seen. The sinusoids are closed. Hæmatoxylin and eosin. ×110.

FIG. 8.—Normal pig no. 1. Normal liver, to show amount of perilobular fibrous tissue, relation of sinusoids to liver cells and distribution of red blood cells (black) within the lobule. Masson's trichrome stain. ×65.

FIG. 9.—Normal pig no. 1. From same section as fig. 8, to show variability in distribution of red blood cells (black) within a normal liver. This represents the maximum concentration of red blood cells observed within normal lobules. Masson's trichrome stain. ×65.

FIG. 10.—Pig no. 11 (group 2). Well-marked central and periportal fibrosis. Hæmatoxylin and eosin. ×36.

FIG. 11.—Pig no. 7 (group 1). Transverse section of a hepatic vein whose lumen measured 6 × 1 mm. From above downwards :—lumen of vein; muscle bundles, mainly longitudinal; perilobular fibrous tissue; liver parenchyma. The middle of the figure shows a tributary of the hepatic vein. Masson's trichrome stain. ×50.

HEPATIC CHANGES IN YOUNG PIGS
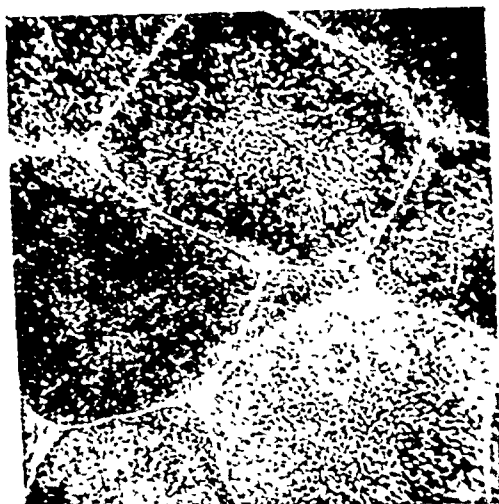
FIG. 6.
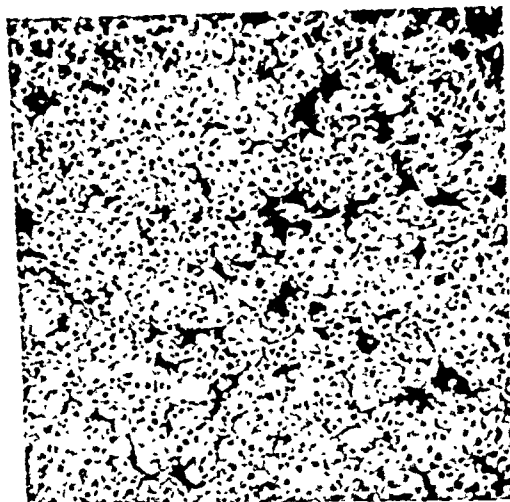
FIG. 7.
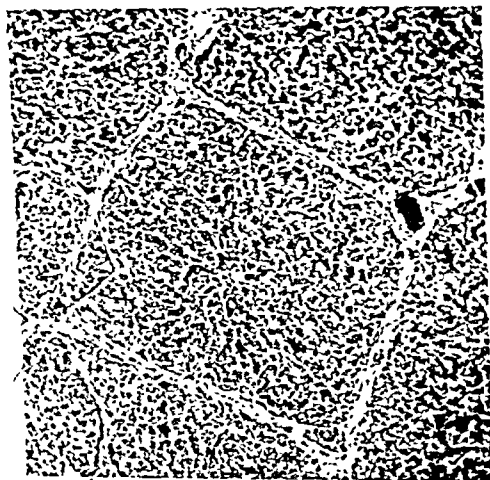
FIG. 8.

FIG. 9.
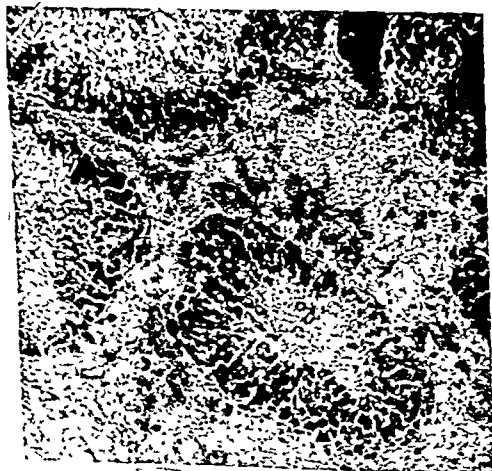
FIG. 10.

FIG. 11.

cold, especially cold applied directly to the abdomen as in a young pig lying on a non-insulated damp floor, may cause derangement of blood flow through the liver as well as through the vessels of the abdominal wall itself.

If liver damage is accepted as the essential pathology of this disease of young pigs, it readily explains the other clinical and post-mortem findings, including the anorexia, ascites and anæmia, and the interval of about 3 weeks between birth and the development of the lesion. The two observed modes of death can also be explained ; the sudden death with cardiac overaction by rapid abrupt increase of venous pressure and the gradual shock-like death from gross derangement of liver structure and function.

The whole picture is complicated and we have insufficient data to answer all the questions that arise. For example we cannot yet say in what manner prolonged exposure to excessive cooling may act, directly or indirectly, on the nutritional state of the animals. It will increase calorie and protein requirements and perhaps also the need for specific nutrients (Smith *et al.*, 1948). The hepatic lesions in our pigs differ from those which Glynn and Himsworth (1944) produced in rats by feeding them on a protein-deficient diet and from those which Gillman *et al.* (1945) produced in rapidly growing young rats given a poor diet of maize-meal porridge and sour milk. At this stage the first need from the practical point of view is to establish that these lesions are somehow connected with a cold environment and should no longer be attributed to iron deficiency.

SUMMARY

1. The disease described by McGowan (1924) as iron-deficiency anæmia of pigs cannot be prevented by giving iron.

2. The essential lesion of the disease is nutmeg liver and its sequelæ.

3. The same clinical and pathological picture can be produced in young pigs by rearing them in a cold and damp environment and prevented by affording them additional warmth.

4. The liver changes observed appear to be explicable on the basis of derangement of the hepatic circulation such as might reasonably be expected to result from prolonged exposure to excessive cooling.

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576 . 852 . 211 : 615 . 778 (Penicillin)

THE EFFECT OF PENICILLIN ON THE TUBERCLE BACILLUS : TUBERCLE PENICILLINASE

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LITERATURE

ILAND (1946) showed that *Mycobacterium tuberculosis* was inhibited by penicillin (20-80 units per ml.) under specified conditions, namely slide-cell culture of fresh tuberculous material in a watery extract of Lowenstein's medium. Using a similar technique Howie (personal communication) also obtained inhibition and Kirby and Dubos (1947) and Solotorovsky *et al.* (1948), using different methods, likewise confirmed the inhibitory effect. The results of both the latter groups of workers agree with Iland's finding that the size of the inoculum is a limiting factor under most conditions of test. The importance of the size of the inoculum was again stressed for other organisms of admitted sensitivity by Luria (1946) and Parker (1946), and in an Annotation (1949). Kirby and Dubos recorded inhibition with as little as one unit of penicillin per ml., depending on the size of the inoculum and the medium. Espersen (1949), with a slide-culture method, also found inhibition of tubercle bacilli by penicillin within the range quoted by Iland. In contrast to these findings Ungar and Muggleton (1946) claimed that the growth of human tubercle bacilli was stimulated by the presence of 1 or 5 units per ml. of penicillin.

Riviere *et al.* (1947) reported that experimental tuberculosis in guinea-pigs was increased in severity by penicillin treatment. This was investigated by Hauduroy and Rosset (1948), who concluded that penicillin had no action on the course of tuberculosis in the guinea-pig and that the effect noted was due to the toxicity of the drug for this animal. Similar reports for other diseases have been published (Herrell, 1945) and it would appear that penicillin is so toxic for guinea-pigs that they must be regarded as unsuitable for experimental work with this substance. The present paper shows that penicillin may not be responsible for the stimulating effect on growth that Ungar and Muggleton observed, and describe the production by a strain of the tubercle bacillus of a penicillin-destroying enzyme-like substance which we have called tubercle penicillinase.

In Ungar and Muggleton's experiments approximately 2×2 mm. pieces of growth of *Myco. tuberculosis* were floated on the surface of a modified Long's medium. To the test cultures 1 or 5 units per ml. of pure penicillin were added and the concentration was maintained by repeated additions of penicillin after assays at 48-hour intervals. The assay results were not quoted. The control

cultures without penicillin showed less growth than the tests, the weighed amounts of growth ranging from 2/3 to 1/5 of the amounts in the test cultures. The cultures were grown in 4-oz. (112 ml.) screw-cap " medical flats " each containing 50 ml. of medium.   This technique resembles that of the Oxford workers (for details see Iland) and of Smith and Emmart (1944), except that these workers used media containing broth or serum and that growth occurred throughout the medium and not as a pellicle.   The conditions necessary for pellicle growth of the tubercle bacillus in synthetic media have been closely studied (see, for example, Corper *et al.*, 1939-40; Cohn, 1944).   The access of air is critical, because the organisms need a liberal supply of oxygen (Novy and Soule, 1925 ; Kempner, 1939).

In Ungar and Muggleton's experiments the volume of air over the culture is 62 c.c. compared with the 500-700 c.c. which would be optimal according to the figures of Novy and Soule.   This fits the fact that the weight of organisms in Ungar and Muggleton's test cultures with penicillin is no more than that obtained by other workers with similar media under optimal conditions (Wong, 1937 ; Corper *et al.*, 1939-40).   Thus Ungar and Muggleton's control cultures without penicillin were probably depressed from lack of oxygen, which was constantly renewed in their test cultures when the caps were removed for the assay and replacement of the penicillin.   An experiment to test this hypothesis was set up.

EXPERIMENTAL

Long's modified medium (Ungar and Muggleton) was distributed in 50 ml. amounts in 4-oz. screw-cap medical flats.   The cultures were inoculated with 2×2 mm. of pellicle from a young culture of the 418 strain as used by Ungar and Muggleton.   The penicillin was the pure sodium salt (Glaxo).   Three sets of cultures were set up :— (1) *Control*.   No penicillin was added and the bottles were not opened

TABLE I

Increased growth of tubercle bacilli from repeated opening of the cultures

Set	Treatment	Growth (visual assessment) of culture no.						Weight of growth (g.)
		1	2	3	4	5	6	
1	No penicillin. Not opened	1	2	1·5	2	2	1·5	1·5
2	No penicillin. Opened every 48 hours	4	3	3	4	4	4	3·0
3	Penicillin, 5 units/ml. Opened every 48 hours	3	4	3	4	4	4	2·8

during the experiment.   (2) *Control*.   As set 1, but the bottles were opened at the same time and for the same duration as the third set of cultures but no penicillin was added.   (3) *Test*.   Five units per ml. of penicillin were added at the beginning of the experiment.   The levels were maintained by assay at 48-hour intervals with the addition

of penicillin as necessary. All cultures were incubated at 37° C. for 3-4 weeks. Four experiments were performed and all gave similar results. Growth was assessed visually as described by Ungar and Muggleton and the cultures were then shaken, filtered off on a Buchner funnel, " dried " by suction for ten minutes and weighed. The results of one experiment are given in table I. The figures indicate that the greater amount of growth in set 3 than in set 1 could be due to a better supply of oxygen and not to the penicillin.

Tubercle penicillinase

During these experiments, assays for penicillin at 48-hour intervals showed that there was complete loss of penicillin after the first few days. Three 15-day-old cultures were therefore assayed at 2-hourly intervals after the addition of penicillin, and it was found that 5 units · per ml. were destroyed in two hours. The sterile medium by itself showed a loss of about 1 unit per ml. in 24 hours at 37° C. It was decided to investigate this phenomenon further to see if a penicillinase was being produced similar to that described for other organisms. The destruction of penicillin by a rapidly growing avirulent strain of *Myco. tuberculosis* was described by Woodruff and Foster (1945).

Methods. The culture fluid to be tested for penicillinase activity was incubated at 37° C. for ½ or 1 hour with various strengths of penicillin. Samples were then taken up on sterile 6-mm. filter-paper discs and tested for the presence of penicillin on culture plates seeded with a strain of the Oxford Staphylococcus. In each case control solutions of penicillin were also tested, using 0·85 per cent. sodium chloride instead of culture fluid. The highest concentration of penicillin destroyed by each preparation was determined. In tables II and III penicillin concentrations destroyed are expressed as units per ml. of penicillin-penicillinase mixture, the volume of the original penicillinase preparation producing the effect being given separately in the table heading.

In all the experiments described, one strain of tubercle bacillus, designated 418, was used. Except in the preliminary experiment the organism was grown on Long's medium in 200-ml. amounts in conical litre flasks or in 350 ml. amounts in round 2-litre flasks stoppered with cotton-wool plugs covered with paper to minimise evaporation but allow a free uptake of oxygen. The cotton wool used had been previously baked at 150° C. for one hour.

Preliminary experiment. Four-weeks-old test and control cultures from one of the experiments described above were filtered separately through Seitz filters. The resulting cell-free fluids were tested for penicillinase activity with negative results. Since the crude culture had shown penicillinase activity, as already described, we decided that filtration might be inactivating the enzyme by adsorbing it and a larger experiment was set up to investigate this and other points.

Penicillinase in a 4-weeks culture. Three hundred and fifty ml. of a 4-weeks-old culture were gently shaken to distribute the pellicle throughout the medium. The culture was then separated into five

parts and the effects of heating and of filtering were tested. The results are shown in table II.

TABLE II

Penicillinase activity in 1·0 ml. of a 4-weeks-old culture of tubercle bacilli

Preparation	Units of penicillin per ml. destroyed in 30 mins. at 37° C.
1. Culture gently shaken to ensure even cell suspension	10
2. As 1, but heated at 57° C. for 1 hr. .	0
3. Filtrate of 1 through filter paper (Whatman no. 1)	10
4. As 3, but heated at 57° C. for 1 hr. .	0
5. Seitz filtrate of 1, unheated . . .	2 (in 60 mins.)

Penicillinase in a 2-months culture. The preparation described in table II did not show great penicillin-destroying activity, and we therefore decided to try an older culture in which a considerable amount of lysis had occurred. This was done because work with other organisms had suggested that lysis liberates the active material (Duthie, 1947). Since autolysis of tubercle cultures does not begin to any important extent until the culture is five weeks old, a 2-months-old culture was used. A crude culture fluid obtained by carefully decanting the culture was divided and tested as shown in table III.

TABLE III

Penicillinase activity in 0·5 ml. of a 2-months-old culture fluid

Preparation	Units of penicillin per ml. destroyed in 60 min. at 37° C.
1. Culture fluid centrifuged until cell free *	50·0
2. Crude culture fluid	50·0
3. As 2, but shaken for 15 min. . . .	17·5
4. As 2, but filtered (Whatman no. 1) .	17·5
5. As 2, but Seitz-filtered . . .	7·5
6. As 2, but heated at 60° C. for 60 min. .	2·5
7. As 2, but boiled for 5 min. . . .	2·5

* 3000 *r.p.m.* for 15 minutes

The effect of hydrogen-ion concentration. Penicillin solutions of 5 and 10 units per ml. were made up in buffer solutions ($M/150$ phosphate) at pH levels 3, 4, 5, 6, 7 and 8. Equal volumes of crude culture fluid (pH 6·5) were added to the test series, giving final pH levels of 3·7, 4·8, 5·7, 6·0, 6·8 and 7·8. To a control series equal volumes of distilled water were added and all were incubated at 37° C. for 1 hour and the penicillin content assayed as already described. The results with 5 and 10 units of penicillin per ml. (figs. 1 and 2)

show that the optimum pH for tubercle penicillinase appears to be 6·0, with a falling off in activity at each end of the scale. The control shows some effect of the pH on the penicillin itself. The graphs were constructed by plotting the size of the inhibition zone in the assay against the pH.
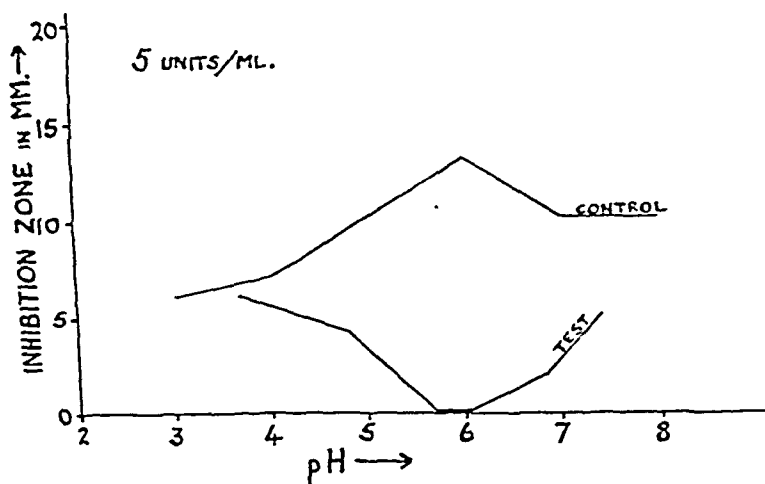
FIG. 1.
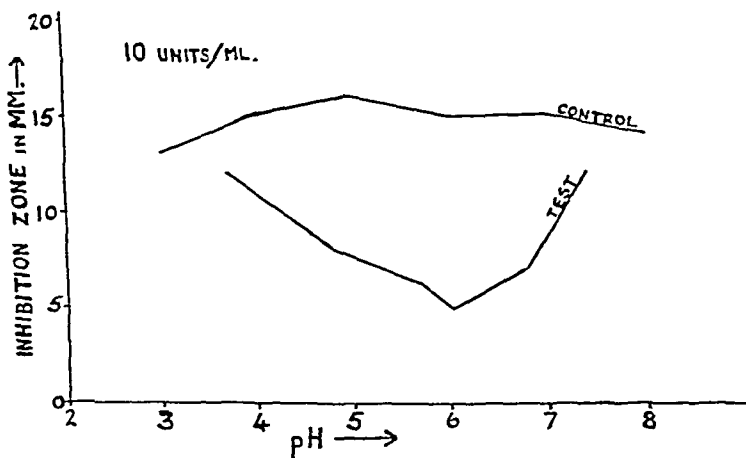
FIG. 2.

FIGS. 1 and 2.—Effect of various levels of pH on the activity of tubercle penicillinase, assayed for inhibitory effect on Staphylococcus on the surface of a culture plate. Control = penicillin solution (5 units/ml. in fig. 1; 10 units/ml. in fig. 2)+distilled water. Test = same penicillin solutions+crude culture fluid from 2-months-old culture of tubercle bacilli.

Extraction of the penicillinase. The active substance was rather unstable, losing its penicillin-destroying power quickly at room temperature. An attempt to obtain a stable preparation by Harper's (1943) method was not a success, as the resulting material had the power to destroy only 6 units of penicillin per ml. in 1 hour at 37° C.

DISCUSSION

The experiments described in this paper show that the strain of tubercle bacillus examined can produce a penicillin-destroying substance which has enzyme-like properties: namely it is unstable to heat, losing nearly all its activity after being heated to 60° C. for one hour; it is adsorbed when a solution containing it is passed through a bacterial filter and to a less extent by ordinary fine filter paper; it is destroyed by shaking; and it works best at pH 6·0, being progressively less active at pH levels above and below this optimum. The adsorption by a Seitz filter of penicillinase from other sources has been observed before (Woodruff and Foster, 1945). The effect of shaking could be an example of the inactivation of an enzyme by adsorption on to the surface of a foam. This could also be the reason for Duthie's failure to obtain a high yield from aerated deep cultures of *Bacillus subtilis*. On the other hand the effect of excessive oxygenation cannot be ruled out, as it is generally agreed that penicillinase is inactivated by increased oxygen tensions and works better in the presence of an agent lowering the redox potential such as thioglycollic acid (Woodruff and Foster, 1945; Sumner, 1948). According to Henry and Housewright (1947) Fe^{+++} ions inhibit penicillinase, and as the medium used in the present investigation contained 0·007 mg./ml. of Fe^{+++} ions, this may be responsible for the low figures for activity which we obtained compared with those found by other workers for different organisms.

Many organisms have been described as producing a penicillin-destroying agent to which the term penicillinase has been applied, although it has not been shown that all the preparations are identical. Organisms of the *subtilis* group produce the most active material, which can be obtained in a stable extracellular form. As the most active preparations are obtained when some, but not complete, lysis has occurred and are not obtained when lysis does not occur (Duthie), it is likely that the enzyme is not extracellular in the strict meaning of the word but is intracellular in the living organism and is liberated only on its death and lysis. Our experience with *Myco. tuberculosis* suggests that this may be the case, as our older, partly lysed cultures gave much more active preparations than younger cultures.

SUMMARY

1. It has been shown that an alleged enhancing effect of penicillin on the growth of the tubercle bacillus is due not to the penicillin, since this is destroyed, but to the improved oxygen supply to test cultures which results from their being frequently opened for penicillin assay.

2. The virulent laboratory strain of tubercle bacillus examined produces a penicillin-destroying substance with enzyme-like properties similar to those described for penicillinase from other organisms

We wish to thank Professor J. F. D. Shrewsbury for his interest in this work.

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576 . 8 . 097 . 3 : 576 . 851 . 21 (Group B)

THE IMMUNISATION OF MICE AGAINST GROUP-B STREPTOCOCCI BY THE INTRAPERITONEAL INOCULATION OF LIVING CULTURES COMBINED WITH PENICILLIN

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IN a previous paper (Pattison, 1948) the active immunisation of mice against group-B streptococci by intraperitoneal inoculation of gradually increasing doses of living whole-broth cultures was described. Further work has indicated that the use of living cultures—especially of unknown strains—requires careful control to avoid killing a high proportion of the mice before immunity has been achieved. It seemed possible that penicillin might be used to control the lethal power of immunising doses of streptococci without seriously diminishing their antigenic activity, and the experiments here reported show this to be the case.

MATERIALS AND METHODS

The group-B streptococcus used in these experiments, strain S 13, has already been fully described (Pattison, 1948, 1949). During the experiments the strain was subcultured each week on Bacto-tryptose ox-blood agar; and whole Todd-Hewitt broth cultures (Todd and Hewitt, 1932) were used for mouse inoculations, all of which were given by the intraperitoneal route. Surface plate counts on Bacto-tryptose ox-blood agar were made of all cultures inoculated.

Penicillin inocula were prepared by dissolving tablets containing 10,500 units of the calcium salt in distilled water. The volume inoculated was 0·5 ml. intraperitoneally in every case. The procaine penicillin G containing 2000 units of penicillin per ml. was obtained through the courtesy of Boots Pure Drug Company.

The test dose of strain S 13 has been 0·5 ml. of an 18-hour whole Todd-Hewitt broth culture containing not less than 100 million living organisms per ml.

EXPERIMENTAL OBSERVATIONS

Treatment with penicillin of mice inoculated intraperitoneally with strain S 13

To determine the efficiency of penicillin in protecting mice previously exposed to strain S 13, mice were inoculated intraperitoneally with a test dose of this strain and half-an-hour later with either a single large dose of penicillin or a series of smaller doses. These tests were repeated several times and sample results are given

in table I. It was concluded that a series of small doses of penicillin was highly efficient in protecting mice against a test dose of this strain.

TABLE I

Results of intraperitoneal treatment with penicillin of mice previously inoculated intraperitoneally with a test dose of strain S 13

Expt. no.	Group	Penicillin	No. of mice	No. living beyond 3 days
1	S 13+ penicillin	One inoculation of 5250 units $\frac{1}{2}$ hr. after test dose	45	6
	Broth+ penicillin	One inoculation of 5250 units $\frac{1}{2}$ hr. after 0·5 ml. broth	20	20
2	S 13+ penicillin	Four inoculations each of 210 units, $\frac{1}{2}$ hr., $2\frac{1}{2}$, $4\frac{1}{2}$, and $6\frac{1}{2}$ hrs. after test dose	20	19
	S 13 only	Untreated	20	0

Treatment of mice with penicillin at varying intervals after exposure to an intraperitoneal test dose of strain S 13

Having demonstrated that a series of small intraperitoneal doses of penicillin would protect mice previously inoculated with a test dose of strain S 13, it was necessary to determine how soon after the test dose penicillin must be injected in order to give protection. Groups of 20 mice were inoculated intraperitoneally with a test dose of this strain, and at varying intervals thereafter were given 3 intraperitoneal inoculations each consisting of 1050 units of penicillin in distilled water. This dose was chosen as being easily prepared by dissolving one 10,500-unit tablet of the calcium salt in 5·0 ml. of distilled water.

TABLE II

Results of intraperitoneal treatment with three 1050-unit doses of penicillin of mice previously inoculated intraperitoneally with a test dose of strain S 13

Group	Injection of penicillin : hours after test dose	No. of mice	No. living beyond 3 days
1	1 : 3 : 5	20	14
2	2 : 4 : 6	20	4
3	3 : 5 : 7	20	3
4	4 : 6 : 8	20	1
5	5 : 7 : 9	20	1
6	Untreated	20	0

The results (table II) showed that, to give efficient protection, the first dose of penicillin must be injected less than one hour after the test dose.

Immunisation of mice by intraperitoneal inoculation of strain S 13 followed by penicillin

On the basis of the evidence so far obtained, it was decided to try immunisation of mice by intraperitoneal inoculation of a test dose of strain S 13 followed by 3 inoculations of 1050-unit doses of penicillin injected half-an-hour, 2 hours and 6 hours after the culture. Further, it was decided to repeat this immunising routine (*i.e.* culture followed by penicillin) at 4-day intervals until immunity could be demonstrated. This experiment was carried out twice and mice were tested for immunity after 2 and after 3 immunising doses. Results (table III) showed evidence of immunity after 2 and of greatly enhanced immunity after 3 immunising doses.

TABLE III

Results of intraperitoneal inoculation with a test dose of strain S 13 of (a) mice previously inoculated at 4-day intervals with test doses of the same strain followed half-an-hour, 2 hours and 6 hours later by intraperitoneal inoculations of 1050 units of penicillin, (b) mice previously inoculated with broth instead of S 13, and (c) untreated mice

Expt. no	No. of preparatory inoculations	No. of days after last preparatory inoculation	Group	No of mice	No living beyond 3 days
1	2	5	S 13+penicillin	10	5
			Broth+penicillin	10	0
			Untreated	5	0
	3	5	S 13+penicillin	10	10
			Broth+penicillin	10	0
			Untreated	10	1
2	2	5	S 13+penicillin	10	7
			Broth+penicillin	5	0
			Untreated	5	0
	3	3	S 13+penicillin	49	49
			Broth+penicillin	14	0
			Untreated	14	1

Immunisation of mice by intraperitoneal inoculation of strain S 13 followed by procaine penicillin G

A drawback to the use of penicillin to control the lethal action of streptococci inoculated as described was that several inoculations of penicillin were required, making the method laborious when large numbers of mice were used. To obviate this, a single inoculation of procaine penicillin G containing 2000 units of penicillin per ml. was used to replace 3 inoculations of the calcium salt of penicillin in distilled water.

To test the efficiency of the procaine penicillin G in controlling the lethal action of a test dose of strain S 13, 10 mice were inoculated intraperitoneally with a test dose of this strain and half-an-hour later with 0·5 ml. procaine penicillin G (*i.e.* 1000 units of penicillin).

Three of these mice died on the third day after inoculation ; the other 7 remained healthy. Five untreated control mice were dead within 24 hours of inoculation, and 5 other mice inoculated intraperitoneally with 0·5 ml. procaine penicillin remained healthy.

To show that mice that had received several inoculations of procaine penicillin G without culture did not develop resistance to streptococci, 5 animals were inoculated intraperitoneally four times at 4-day intervals with 0·5 ml. procaine penicillin ; 2 days after the last inoculation they were exposed to an intraperitoneal test dose of strain S 13. All died within 24 hours.

The results of these experiments showed that (a) a single intraperitoneal dose of 1000 units of procaine penicillin G caused no evidence of disease in untreated mice and controlled to a considerable extent the lethal action of an intraperitoneal test dose of strain S 13, (b) four intraperitoneal doses of procaine penicillin G gave no protection to mice exposed 2 days after the last dose to strain S 13.

An effort was then made to immunise 100 mice by inoculation of a test dose of strain S 13 followed half-an-hour later by a single dose of 0·5 ml. procaine penicillin G. This procedure was repeated after an interval of 7 days, and 7 days later immunity was tested by exposure of all the mice to an intraperitoneal test dose of the same strain. Good immunity was demonstrated (table IV). It will be noted that only

TABLE IV

Results of intraperitoneal inoculation with a test dose of strain S 13 of mice previously immunised by 2 inoculations at a 7-day interval of test doses of the same strain followed half-an-hour later by the intraperitoneal inoculation of 1000 units of procaine penicillin G. The final test dose was given 7 days after the second immunising dose

Group	No. of mice	No. living beyond 3 days
Immunised . .	80	78
Untreated . . .	10	0

80 of the original 100 mice were exposed to the final test dose ; 19 animals had died after the first immunising dose (2 on the 1st day, 6 on the 2nd, 7 on the 3rd, 3 on the 4th, 1 on the 5th), and 1 on the 3rd day after the second immunising dose. The lethal power of the immunising doses of S 13 was further shown by the death within 24 hours on each occasion of 5 mice not treated with procaine penicillin.

The interference of penicillin with the immunising activity of strain S 13 inoculated intraperitoneally

It seemed possible that in arresting the lethal power of streptococci penicillin might also reduce their immunising activity. To test this

possibility, 2 groups each of 40 mice were inoculated intraperitoneally with 1/25th of a test dose of strain S 13 made up to 0·5 ml. with normal saline. Half-an-hour later one group received an intraperitoneal inoculation of 1000 units of procaine penicillin; the other group received no penicillin. Two of the mice not treated with penicillin died on the 3rd day after inoculation and another on the 6th day; all the penicillin-treated mice remained healthy.

Seven days after this inoculation all the mice were injected with 1/10th of a test dose; as before, one group was treated with procaine penicillin and the other left untreated. One untreated mouse died on the 3rd day after this inoculation and another on the 5th day; one penicillin-treated mouse died on the 5th day.

Five days after this second inoculation, 10 mice from each group were exposed to a test dose of strain S 13. Two of the group without penicillin and 6 of the group with penicillin died within 3 days (table V), suggesting somewhat better immunity in the former group.

TABLE V

Results of intraperitoneal inoculation with a test dose of strain S 13 of (a) mice previously inoculated intraperitoneally at 7-day intervals with gradually increasing doses (see text) of living streptococci of the same strain, (b) mice previously inoculated intraperitoneally at 7-day intervals with gradually increasing doses (see text) of living streptococci of the same strain, followed half-an-hour later by 1000 units of procaine penicillin G, (c) untreated mice

Test no.	Group	No. of immuni-ing inoculations	Days after last immuni-ing inoculation	No. of mice	No living beyond 3 days
1	Culture without penicillin	2	5	10	8
	Culture with penicillin	2	5	10	4
	Untreated	...	...	5	0
2	Culture without penicillin	3	3	10	10
	Culture with penicillin	3	3	10	10
	Untreated	...	...	10	0
3	Culture without penicillin	3	6	15	13
	Culture with penicillin	3	6	18	18
	Untreated	...	...	15	0

Seven days after the second immunising inoculation, all the remaining mice received a third intraperitoneal inoculation consisting of half a test dose of strain S 13; as before, one group was treated with procaine penicillin and the other left untreated. One penicillin-treated mouse died 6 days after this inoculation.

Three days after this third inoculation, 10 mice from each group were exposed to a test dose of strain S 13; none died (table V). Six days after the third inoculation, all mice not yet tested were exposed to a test dose of strain S 13; 2 animals died in the group without penicillin, but all the penicillin group survived (table V).

It is concluded that the use of procaine penicillin in this experiment interfered only slightly—if at all—with the immunising activity of the streptococci inoculated.

DISCUSSION

Pre-requisites for the successful use of the immunisation method described are a knowledge of the lethal power of the strain used and of the dose or doses of penicillin required to control it. When mice are the test animals employed, the loss of a small percentage during the immunising process is of little consequence, but with larger animals more careful control would be required. The success of the method appears to depend on a nice balance between the lethal power of streptococci and the protective power of penicillin. It would appear also that, without any important reduction of immune response, death of animals undergoing immunisation may be prevented by the use of larger and more frequent doses of penicillin.

CONCLUSIONS

1. The mouse lethal effect of an intraperitoneal test dose of group-B streptococcus strain S 13 was controlled by 3 intraperitoneal inoculations, each of 1050 units of the calcium salt of penicillin in distilled water, given half-an-hour, 2 hours and 6 hours after the test dose. The same test dose was also largely controlled by a single inoculation of 1000 units of procaine penicillin G given half-an-hour after the test dose.

2. Repetition at 4-7-day intervals of this test dose-penicillin routine created active immunity in mice.

3. Penicillin used as described had little detectable deleterious effect on the immune response of inoculated mice.

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576 . 8 . 093 . 31 : 616—008 . 843 . 5

A SCREENING PLATE FOR THE RAPID IDENTIFICATION OF FÆCAL ORGANISMS *

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(PLATE LXXX)

THE bacteriologist engaged in examining large numbers of fæcal specimens often finds it difficult to combine speed with accuracy. The modern use of efficient selective media has made it essential to exercise great care in investigating suspicious colonies found on the primary plates. Colonies of pathogens on such media as desoxy-cholate-citrate agar (Leifson, 1935; Hynes, 1942), eosin-brilliant green agar (Jones, 1936) and Wilson and Blair's medium (Wilson and Blair, 1931) are frequently contaminated with other organisms. These may be visible as minute colonies which may perhaps be avoided with care, or they may be altogether invisible. Their growth is in fact suppressed by the selective agent in the primary plate but they grow freely on subculture in non-inhibitory media. To inoculate tubes containing liquid media directly from colonies on such primary plates is unsound and invites the unfortunate consequences which so often follow.

Yet the bacteriologist's dilemma is real. If he insists on accuracy at all costs, his reports may be several days too late to be of any practical value ; if he sacrifices accuracy in the attempt to give a rapid report, he may find that his so-called short-cuts are disastrous. This paper describes a " screening " plate which has been devised in an attempt to overcome these difficulties. The choice of the best primary media for bacteriological examination of fæces is another matter. I am concerned here only with the methods used in picking and further investigating suspicious colonies seen on primary plates.

Methods commonly used

The table shows some of the methods commonly used.
Method I is accurate but slow.
Method II appears to have the advantage of giving rapid results though the actual inoculation of many sets of sugars is time-consuming, but the method is unsound, since the sugar reactions may be vitiated by small numbers of undetected contaminating organisms.

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* A report to the Medical Research Council.

Method III certainly gives considerable information and eliminates many non-pathogens at the earliest possible moment, but it is rather cumbrous and is still open to the criticisms applied to method II.

TABLE

Methods commonly used for picking single colonies from primary plates

Days	Method I	Method II	Method III	Method IV
1	Inoculate MacConkey's lactose agar	Inoculate peptone water Incubate 6-8 hrs. Inoculate sugars	Inoculate MacConkey's lactose agar and MacConkey's sucrose agar and sugars (short set)	Inoculate double or triple sugar slope, sometimes with indicator of H_2S production
2	Examine plates Agglutinate Inoculate sugars	Read sugars Agglutinate	Check purity Read sugars Agglutinate Inoculate further sugars if necessary	Agglutinate Inoculate sugars
3	Read sugars		Read further sugars	Read sugars

Method IV (Russell, 1911-12; Krumwiede and Kohn, 1917-18; Kligler, 1917; 1918; Bailey and Lacy, 1927; Sulkin and Willett, 1939-40). These multiple sugar slopes are informative but it is impossible to be certain of the purity of the cultures.

There are, of course, many other methods in common use, but most of them probably fall into one or other of these four groups. Each of them has serious disadvantages and the problem was to devise a medium which would combine speed with accuracy.

THE SCREENING PLATE

The medium

This consists really of two media. Fig. 1 shows diagrammatically the appearance of the plates. Medium A is poured first (about 10 ml. quantities) into Petri dishes inclined at such an angle that the medium occupies a quarter to a third of the area of the Petri dish. This medium is allowed to set, the dishes are then laid flat and 10-15 ml. of medium B poured. When this has set the plates are dried at 37° C. for 1-2 hours and are ready for use. For composition of media see appendix (p. 351) and legend to fig. 1.

The method of inoculation is illustrated diagrammatically in fig. 2. A single colony is picked with a straight wire from the primary plate and inoculated heavily along the centre of the screening plate as shown. The inoculum is then spread out so as to obtain separate colonies on both sections of the medium and on both sides of the plate. Immediately after inoculation two sterile glass coverslips are dropped on to the medium as shown and then two sterilised strips of blotting paper, the first containing mannitol, the second sucrose. The plates are then incubated at 37° C. and examined next day.

Fig. 2.—Screening plate (4-in. Petri dish) after inoculation.

A = lead acetate-thiosulphate-agar; B = desoxycholate-lactose-agar; C = cover-slips; M = 50 per cent. mannitol strip; S = 50 per cent. sucrose strip. The vertical lines indicate the method and direction of inoculation.

Fig. 1.—The screening plate. × ⅔.

Composition of media (pH 7·6)

	Per cent.
A. Lead acetate	0·05
Sodium thiosulphate	0·125
Agar	2·0
Tryptic digest broth (full strength)	
B. Lactose	1·0
Sodium desoxycholate	0·25
Neutral red	0·0025
Agar	2·0
Tryptic digest broth (half strength)	

Examination of the plates

Fig. 3 shows diagrammatically the information which can be obtained from examination of the cultures the following day. The whole plate is inspected for purity of the culture. Single colonies are available for inspection of colonial form, for slide agglutination tests and for carrying out further sugar reactions if necessary. On section A of the plate spreading strains of *Proteus mirabilis* and *Proteus vulgaris*
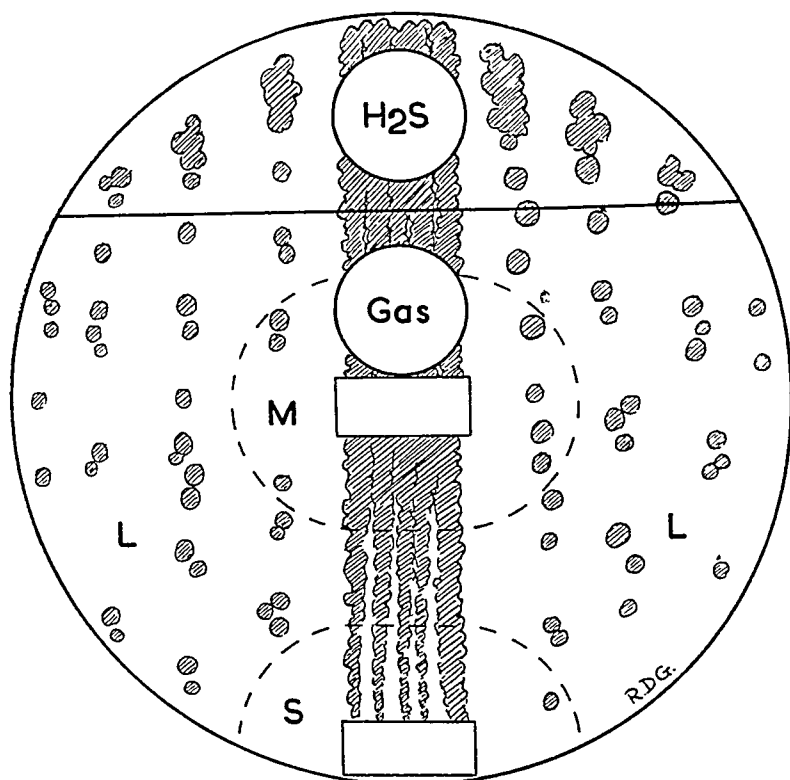
Fig. 3.—Screening plate after overnight incubation.
M and S = areas of reddening and precipitation around mannitol and sucrose strips;
L = area in which reddening and precipitation indicate lactose fermentation.

spread freely and Pseudomonas shows strong pigment production. H_2S production is shown by blackening under the cover-slip; separate Salmonella colonies may also be brown. *Shig. sonnei*, *Shig. flexneri* and other non-H_2S producers give clear-cut negative results. Many cultures (*e.g. Shig. flexneri*, Proteus, Pseudomonas and some paracolon bacilli) are easily recognised by their characteristic smell which is well developed. On section B of the plate lactose fermenters give red colonies with precipitation of the desoxycholate; mannitol fermentation is indicated by reddening and precipitation extending for 15-20 mm. around the mannitol strip of blotting paper, and sucrose

PLATE LXXX

RAPID IDENTIFICATION OF FÆCAL ORGANISMS
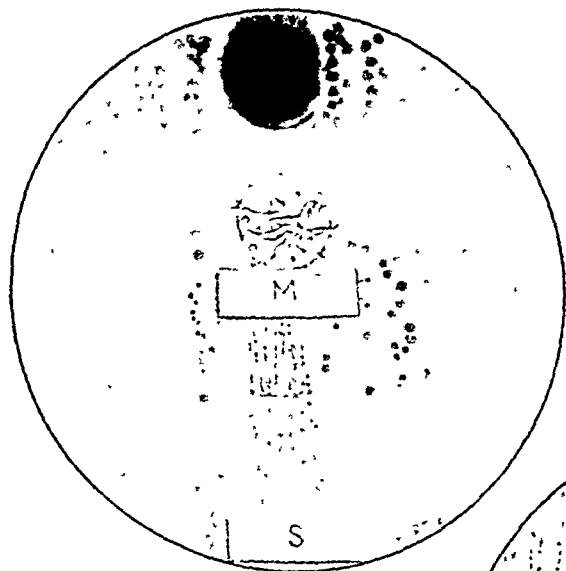
FIG. 4.—*Salmonella paratyphi* B.

FIG. 5.—*Shigella sonnei.*
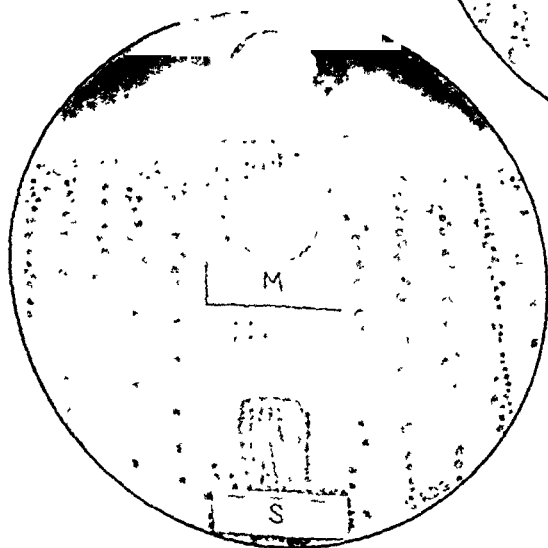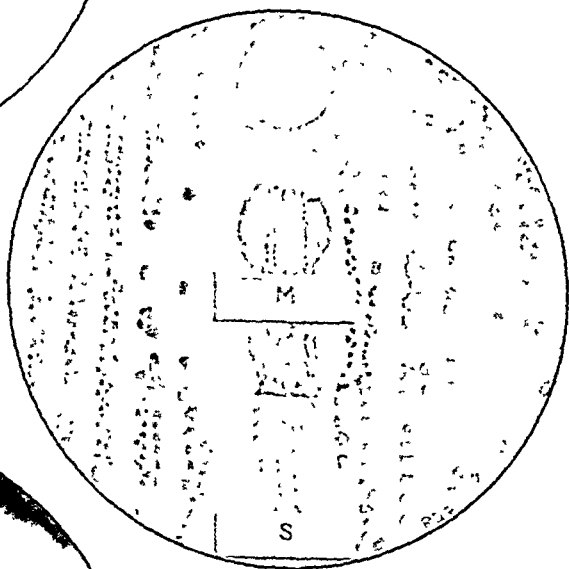
FIG. 6.—*Proteus vulgaris.*

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fermentation similarly around the sucrose strip. Gas production from mannitol is indicated clearly and unequivocally by bubbles of gas or fissures in the agar underneath the second coverslip. Figs. 4, 5 and 6 are colour drawings of plates inoculated with *Salmonella paratyphi* B, *Shigella sonnei* and *Proteus vulgaris* respectively.

DISCUSSION

The medium was devised with a strictly practical end in view. It is generally agreed that in examining fæces for pathogenic organisms a variety of selective media must be used if the best results are to be obtained, since the perfect selective medium has yet to be devised. The examination of each specimen may therefore involve studying the biochemical and serological reactions of a number of suspicious colonies from primary plates. Any medium or method, therefore, which introduced order and precision into the cumbrous and some-times almost chaotic process of examining large numbers of racks of sugar tubes in various stages of incubation and purity would be of considerable practical value. There are, however, a few points, some of practical some of theoretical interest, which deserve mention here.

In medium A the formula described (see appendix) gave the best indication of H_2S production. Sodium thiosulphate, lead acetate, full-strength tryptic heart base and the coverslip all appear to be essential. But neither the mechanism of the blackening nor the source of the H_2S can be regarded as clear. A possible suggestion is that the coverslip delays the escape of H_2S and enables it to be trapped as lead sulphide by the lead acetate, but it is doubtful whether the source of the H_2S is organic sulphur (*e.g.* cysteine) in the tryptic heart-broth agar or the added thiosulphate, or whether perhaps one of these catalyses the production of H_2S from the other.

In medium B various modifications in the base were tested. Half-strength tryptic heart broth in 2 per cent. agar was found to be the most satisfactory. When full-strength broth was used in the agar, enough acid could be produced to give a pink colour under a coverslip even in the absence of added carbohydrate. Production of acid with or without gas from carbohydrates or alcohols on the surface of agar plates is very different from fermentation in liquid media under anærobic or semi-anærobic conditions. When in addition the carbohydrates are placed in blotting paper strips on a small section of a plate diffusion becomes important. The sharpness and the extent of the zone in which acid production is indicated depend on a balance determined largely by the amount of fermentable substrate, its rate of utilisation, the speed with which it or its acid products diffuse into the surrounding medium and the alkalinity produced by further bacterial growth.

At first, strips of filter paper soaked in sugars were tested.

Cruickshank (1948) showed that strips of filter paper soaked in dyes could be successfully used in the differentiation of Brucella cultures, so strips of filter paper soaked in sugars were tested on the screening plates. But it was found that whereas acid production was clearly indicated by separate colonies, with confluent or semi-confluent growth acid produced in the early hours of growth disappeared on overnight incubation. For this reason strips of blotting paper were used as a convenient method of obtaining a reservoir of sugar large enough to give continued acid production in overnight cultures. The sugar reactions obtained on the screening plates have shown perfect correlation with reactions obtained in the conventional peptone-water tubes : a few sucrose fermenters have given positive results overnight on the plates but have taken several days to ferment sucrose in peptone-water tubes.

The use of the coverslip to indicate gas production was suggested to me by H. A. Tarr (personal communication, 1948). Gas production is shown mainly by fissuring of the agar and sometimes by bubbles of gas under the coverslip as well. The coverslip is a reliable indicator of gas production and the results give perfect agreement with conventional tests in peptone-water sugars containing Durham tubes. Gas producers often give bubbles of gas but no fissuring of the agar underneath the " H_2S " coverslip on the other part of the plate, whether they are H_2S producers or not. The source of this gas may be fermentable substances in the full-strength tryptic heart agar on this part of the plate, but these bubbles of gas cause no difficulty and can be safely ignored.

Desoxycholate has proved in this medium to be invaluable, not as a selective agent but as an aid in differentiation. The concentration used (1 in 400) is half the concentration in Leifson's medium and in Hynes's modification (Leifson, 1935 ; Hynes, 1942). This concentration has no inhibiting effect on any members of the Shigella or Salmonella genera so far encountered. Most non-pathogens also grow on it freely. This of course was intended, since the medium was meant to reveal latent contaminants not detectable on primary selective plates. The medium has proved remarkably sensitive in showing up small numbers of lactose or sucrose fermenters which cannot be recognised on a MacConkey plate in the presence of a heavy inoculum of non-fermenters. This greater sensitivity in detecting small numbers of fermenters is undoubtedly due mainly to the relatively irreversible precipitation of desoxycholate at low pH ; small amounts of acid are trapped in the colony and do not readily diffuse into the surrounding medium. Somewhat unexpectedly, it was found that many strains of Proteus are markedly inhibited on some batches of medium, especially where the inoculum is not very heavy. These strains are inhibited far more than on Hynes's modification of Leifson's citrate-desoxycholate medium.

Agglutination reactions are reliable on both parts of the plate.

Preliminary *slide-agglutination* results can be accepted with the usual reservations and tube agglutinations are also satisfactory. The sticky growth which often occurs on desoxycholate medium containing citrate has never been observed on either the desoxycholate or lead acetate part of this medium.

The medium is simple and easy to prepare and it keeps well. Even though it is obvious that on storage changes must occur in the concentration of different reagents by diffusion from one part of the medium to the other, these changes do not affect the performance of the ready-made medium up to several weeks.

The method of pouring one part of the medium with the plate at an angle and the rest with the Petri dish flat was adopted as a simple way of obtaining a composite medium and avoids all the complications which *would be involved in cutting partitions in a* plate, cutting out gutters and filling them with agar and so on, with all the difficulties of obtaining a smooth level surface which such manœuvres entail. At the same time the composite medium has all the advantages given by a single plate and the process of picking from primary plates is greatly simplified.

The medium differs from the usual " multiple " media in two important points. (1) It is possible, from the information given by the plates, to divide non-pathogens as well as pathogens into broad groups—non-pathogens are not dismissed indifferently without further characterisation. In many intestinal infections it is quite common to isolate organisms of doubtful pathogenicity, and it is much easier with the screening plate than with multiple sugar tubes to observe *whether, for example, a particular paracolon-like organism is occurring* in a series of epidemiologically related cases. (2) The use of plates on which the purity of the culture can be checked gives an accuracy of diagnosis which is impossible when tubes are used, however carefully colonies are picked.

The medium is flexible and can be modified to meet special circumstances by using other sugars besides or instead of mannitol and sucrose without altering the actual composition of the medium. The exact formula of the medium could no doubt be improved, perhaps, for example, by using other methods of indicating H_2S production. The formula here described has proved consistently satisfactory, but the purpose of this paper is to emphasise the principle rather than the details of the medium. The results of its routine use in combination with rapid urea and rapid indole tests are described in a separate paper (Cook and Knox, 1949).

In conclusion, the medium has achieved the original purpose for which it was devised and its use has in addition brought out some interesting points in the biochemical behaviour of intestinal pathogens and non-pathogens.

SUMMARY

A screening medium has been devised, suitable for the rapid identification of pathogens and rapid elimination of non-pathogens in the routine bacteriological examination of fæces.

The medium is poured in Petri dishes in two parts. Part A, consisting of lead acetate, sodium thiosulphate and full strength tryptic heart broth in 2 per cent. agar, is poured with the Petri dish inclined at a slight angle. When this has set part B, consisting of sodium desoxycholate, lactose, neutral red and half-strength tryptic heart broth in 2 per cent. agar, is poured with the Petri dish flat.

After the plate has been inoculated two glass coverslips and two strips of blotting paper soaked, one in 50 per cent. mannitol and the other in 50 per cent. sucrose, are placed on the heavily inoculated part of the plate.

After overnight incubation the medium indicates spreading of Proteus, pigment production of Pseudomonas, sucrose and lactose fermentation, fermentation of mannitol with or without gas production, and production of H_2S.

The purity of the cultures can be readily checked, the characteristic smell produced by many intestinal organisms is well developed on the medium and single colonies are available for inspection of colony form and for serological investigation.

The coverslip placed near the mannitol slip indicates accurately gas production, while the coverslip on the lead acetate-thiosulphate part of the medium gives clear-cut indication of H_2S production.

The medium is easy to prepare, keeps well on storage and gives rapid and accurate results.

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APPENDIX

Details of the media

Medium A

Prepare 2 per cent. agar with tryptic digest heart broth in the usual way. Adjust pH to 7·6. Bottle in 400-ml. lots. Store in a cool place or the cold room. For use, melt 400 ml. by autoclaving at 10 lb. for ½ hr. Cool on bench. Place in water-bath at about 50° C. for ½ hr. Add 2 ml. of 10 per cent. lead acetate solution and 2 ml. of 25 per cent. (approximately molar) sodium thiosulphate. Adjust pH to 7·6.

Lead acetate solution. Prepare a 10 per cent. solution of lead acetate in water and steam for 1 hr. Allow to settle overnight. The clear supernatant fluid is used. Crystalline lead acetate is preferable to the basic salt.

Sodium thiosulphate solution. Prepare a 25 per cent. solution of sodium thiosulphate. Steam for 1 hr.

Medium B

Prepare 2 per cent. agar made with 50 per cent. tryptic digest heart broth in water. Adjust pH to 7·6. Bottle in 400-ml. lots. Store in a cool place or cold room. For use, add 4 g. of lactose to 400 ml. of the agar. Melt. Place in water-bath at 50° C. for ½ hr. Add 10 ml. of 10 per cent. sodium desoxycholate solution. Adjust pH to 7·6. Add 0·5 ml. of 2 per cent. neutral red solution.

Sodium desoxycholate solution. Ten per cent. solution of sodium desoxycholate as used in Hynes's modification of Leifson's desoxycholate-citrate medium (Hynes).

Neutral red solution. Two per cent. solution in water steamed for 1 hr.

Pouring of the plates

Sterile Petri dishes are fixed in a sloping position on the bench by a suitably inclined tray. 8-10 ml. of medium A are poured into each Petri dish and allowed to set. The dishes are then laid flat on the bench and 10-15 ml. of medium B poured.

Blotting-paper strips

Ford's blotterettes (Ford 428 Mill) were used. It is essential that any paper used must not inhibit bacterial growth. Pieces of blotting paper are soaked in a hot 50 per cent. solution of the appropriate sugar, prepared by steaming for 20-30 minutes, and then hung up to dry for 5-10 mins. in a hot-air oven at 120° C. Each piece is then cut so as to give finally a number of small strips about 20×7 mm. Before starting to prepare the strips it is possible by appropriate spacing to type the first letter of the sugar to be used in several rows so that at the end each strip has its appropriate letter, typed on it (*e.g.* S for sucrose, M for mannitol). The strips are then put into $5 \times \frac{3}{4}''$ test-tubes and autoclaved for 10 mins. at 10 lb. They are then ready for use and can be removed as required with sterile forceps.

Coverslips

Circular glass coverslips no. 1, $\frac{5}{8}''$ diam., or no. 1a, $\frac{3}{4}''$ diam., are thoroughly cleaned with spirit, allowed to dry, placed in a Petri dish and sterilised in a hot-air oven at 180° C. for 1½ hrs. The coverslips can be arranged in such a way that they can be conveniently removed with sterile forceps when ready for use.

Forceps

It is an advantage to have a delicate pair of forceps so that these can be rapidly sterilised in a Bunsen flame.

616—008 . 843 . 5 : 616—008 . 87

BACTERIOLOGICAL EXAMINATION OF FÆCES *

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For some time we have been trying in this laboratory to develop methods which would simplify, shorten and make more precise the routine bacteriological examination of fæces. The value of a rapid test for urea-splitting has been emphasised by Cook (1948), using the lightly buffered medium of Christensen (1946), and a screening plate for the rapid characterisation of pathogens and non-pathogens has been described by Knox (1949). The present communication describes the results obtained during the last few months by the combined use of these two methods with a third, a rapid test for indole, which is briefly described below.

METHODS

A large loopful from a broth suspension of fæces, if solid, or from the untreated fæces if liquid, was inoculated on to Hynes's modification of Leifson's desoxycholate-citrate agar and Wilson and Blair's medium (direct primary plates), and also into Kauffmann's tetrathionate broth and selenite F, from which, after overnight incubation, loopfuls were plated on to the same two solid media (indirect primary plates). After overnight incubation, primary plates were carefully examined and where necessary suitable colonies were tested by slide agglutination with appropriate sera, though the results were always interpreted with caution.

Screening plate

Representative single colonies of all non-lactose fermenters on desoxycholate plates and of all salmonella-like organisms on Wilson and Blair's medium were each inoculated with a straight wire on to a screening plate (Knox). Two sterile cover-slips and two strips of blotting paper, one containing mannitol and the other sucrose. were dropped on to the plate, which was then incubated overnight.

Rapid urea test

Christensen's medium (1946) was used as described by Cook (1948). The medium was inoculated from the screening plate and cultures incubated at 37° C. in a water-bath and the results read after one hour.

Rapid indole test

One ml. quantities of 2 per cent. peptone water were inoculated, not from primary plates but from pure cultures on screening plates. Very heavy inocula were used so as to form in fact thick suspensions in the tubes, which were then

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J. PATH. BACT.—VOL. LXI   353

incubated in a water-bath at 37° C. After one hour they were removed and tested for indole by the usual method of running about 1 ml. of ether down the tube, shaking, and then adding a few drops of Ehrlich's reagent.

Full sets of sugars were inoculated when necessary and consisted of tubes of glucose, mannitol, maltose, lactose, sucrose, salicin and dulcitol peptone water, a plain peptone-water tube, a tube of heart broth containing a lead acetate paper and an agar slope.

General procedure

The cultures on the screening plates were examined after overnight incubation and their purity checked by carefully examining both the heavy growth in the centre of the plate and separate colonies. Further investigation of the cultures depended upon the information given by the plates. A number of pathogens could be immediately recognised and confirmed by slide agglutination, followed by full biochemical and serological investigation where necessary. A number of non-pathogens could also be immediately recognised and eliminated. A further considerable number was shortly afterwards discarded as a result of a single test, e.g. the rapid urea or rapid indole test. The remaining cultures— a small proportion of those picked—required inoculation into a full set of sugars and perhaps serological investigation.

RESULTS

During the period covered by this investigation (July-November 1948) 1077 colonies were examined from 583 of approximately 900 specimens of fæces. All colonies were picked directly on to screening plates and no inoculations were made into liquid media from primary plates. The great majority of the 1077 colonies picked gave a pure growth on the screening plate when examined after overnight incubation. Twenty-six cultures were obviously mixed and a few others showed a small number of lactose-fermenting contaminants in the confluent part of the growth. Of the pure cultures, 90 were picked from Wilson and Blair's medium and 961 from desoxycholate-citrate agar. The latter have been subjected to a detailed analysis, the results of which are shown in table I.

TABLE I

Investigation of 961 colonies picked from desoxycholate citrate agar

Group	Classification	Number of cultures	Total
I	Shigella	99	176
	Salmonella	77	
II	Lactose fermenters	153	514
	Sucrose fermenters	147	
	Ps. pyocyanea	41	
	Proteus (spreading)	173	
III	Proteus (non-spreading)	90 ⎫263	177
	Paracolon bacilli H₂S −, indole + . .	87 ⎭	
IV	Paracolon bacilli, H₂S −, indole − . .	42 ⎫161	94
	,, ,, H₂S + . . .	32 ⎭	
	Miscellaneous non-pathogens . . .	20	

Group I

One hundred and seventy-six pathogens all gave their characteristic pattern on the screening plate, on which, in fact, they were recognised as such and confirmed immediately by slide agglutination. The following Salmonella and Shigella organisms were isolated :— Salmonella 77 (*S. typhi-murium* 50, *S. anatum* 12, *S. newport* 14, *S. bovis-morbificans* 1), Shigella 99 (*Sh. sonnei* 93, *Sh. flexneri* V 1. *Sh. flexneri* 103 5).

S. typhi has also been recognised by its biochemical reactions on the screening plate on a number of occasions in routine work since this investigation was completed.

Group II

By simple inspection of the plates after overnight incubation it was found possible to discard a further 514 of the 961 original cultures as non-pathogens because of lactose or sucrose fermentation, pigment production or spreading. The relatively large number of colonies which fermented lactose on subculture is partly due to the fact that the primary desoxycholate-citrate agar plates were usually examined after less than a full twenty-four hours' incubation.

Group III

A further 177 cultures could be discarded within an hour or two of examining the plates. These consisted of 90 Proteus cultures eliminated by the rapid urea test and 87 paracolon bacilli which could be eliminated by the rapid indole test.

Proteus cultures. The majority of Proteus strains were betrayed by the spreading shown on the lead acetate section of the screening plate. These cultures have already been referred to under group II and they could be at once eliminated without further investigation. Non-spreading strains, however, were not immediately recognised, though their identity was usually suggested by their reactions and characteristic smell. These cultures formed the majority of the group of non-spreading non-mannitol-fermenting strains and weak mannitol fermenters suspected of being *P. rettgeri*, on which it is suggested that a rapid urea test should be done (table II). Of 105 such cultures so tested, it was thus possible to identify 90 as Proteus within an hour or two of examining the plates.

Though inspection of the plates made it possible to assign most of the 263 Proteus cultures (spreading and non-spreading) to their correct biochemical group, for the purpose of this investigation all but 8 strains were classified by any necessary additional biochemical reactions as follows :—*P. vulgaris* 86, *P. mirabilis* 103, *P. morganii* 29, *P. rettgeri* 37. From each of 18 specimens of fæces two different species of Proteus were isolated, all except one of the six possible

combinations being observed. In addition, three different species were obtained from each of three specimens.

Paracolon bacilli. There were 161 organisms in this ill-defined group which failed to ferment lactose and sucrose but fermented mannitol with gas production on the screening plate. There were only 32 of these which were H_2S-positive and whose reactions on the plate therefore resembled those of the Salmonella group; such cultures required full biochemical and serological investigation and are considered under group IV. The remaining 129 cultures failed to produce H_2S and were therefore unlikely to belong to the genus Salmonella. The rapid indole test was reserved for these strains and proved particularly valuable as approximately two-thirds were indole-positive. Eighty-seven in fact could be eliminated by the rapid indole test, while the remaining 42 indole-negative cultures required further biochemical investigation and were placed in the following group.

Group IV

There remained only 94 cultures which could neither be diagnosed as probable pathogens nor eliminated with confidence as non-pathogens within an hour or two of examining the plates. These were the only cultures which required further fermentation tests, except for the pathogens for which biochemical confirmation was necessary. Slide agglutination was also done on cultures whose characters on the plate (biochemical reactions, colonial appearances, smell, etc.) particularly suggested the possibility of a pathogen, *e.g.* the group of 32 H_2S-positive paracolon bacilli.

Agglutination

The screening plate gave reliable slide agglutination with a large number of Salmonella and Shigella cultures. The growth on the lead-acetate section was used for making alcoholised " O " Salmonella suspensions and mercuric iodide suspensions of Shigella cultures. Tube agglutinations were done on 44 strains and the results were always perfectly satisfactory. Suspensions for " H " agglutination were prepared by inoculating a broth tube from the plate early in the day, incubating in a water-bath at 37° C. for a few hours and then formolising the culture.

Rapid indole test

Cultures have been investigated in parallel by this short test and by the usual method of overnight incubation of ordinary peptone-water cultures (5 ml. quantities) in $6 \times \frac{5}{8}''$ tubes. Two hundred and thirty-one cultures were tested; 163 of these gave negative results in 1 hour and after overnight incubation, 61 were positive in both tests, and 7 which gave a negative result at 1 hour were positive after overnight incubation. These last cultures were all strains of *P.*

morganii except for one paracolon culture; they gave a positive result when re-tested by the rapid test after 3 hours' incubation. No false positive results have been recorded.

Peptone water (Eupeptone) has proved as satisfactory a substrate for the rapid indole test as for the orthodox longer test. Tryptic heart broth, although it contains small amounts of sugar, and enzymic casein digest have also proved satisfactory. Arnold and Weaver (1948) have independently described a rapid indole test which is essentially the same as the one described here. They also found that tryptophane in weak peptone-phosphate water provided a suitable medium. The rapid test was designed to make use of the tryptophanase already present in the cultures to be tested.

DISCUSSION

The method described combines the advantage of uniformity in the first step (picking representative samples of all suspicious colonies on to a screening plate) with the flexibility which is necessary and desirable in the later stages of investigation. A large number of cultures (over 50 per cent. of our series) can be immediately excluded from further investigation with the assurance that the cultures are pure and attention can be promptly focussed on presumptive pathogens. Positive slide agglutination will reinforce the biochemical reactions and the bacteriologist is enabled to take immediate steps to telephone a presumptive positive result. When indicated, suspensions for tube agglutination can be prepared and further sugars inoculated without delay. The rapid urea test and rapid indole test enable the bacteriologist to identify a number of the remaining cultures as non-pathogens within an hour or two of inspecting the plates. The number of cultures remaining undiagnosed and requiring further biochemical investigation is reduced to a minimum and sugar sets can be inoculated from a single colony with the reasonable expectation of a pure inoculum.

One of the advantages claimed for the use of a plate of this type for primary subculture (Knox) is the greater opportunity it offers for the detection of mixed cultures and this has proved to be the case. Even with a mixture of two non-lactose-fermenting cultures, a careful study of colonial appearances and biochemical reactions usually gives sufficient information to enable a presumptive diagnosis of both strains to be made. Single colonies are available for slide agglutination and other investigations if these are indicated.

A suggested method for employing the combination of screening plate, rapid urea and rapid indole tests is summarised in table II. The use of a single medium on to which all suspicious colonies are plated simplifies the work of both the technician inoculating the cultures and the bacteriologist examining the results. The technique of inoculating the plates and placing coverslips and " sugar strips " is quickly learnt and a series of plates can be inoculated more rapidly

than an equivalent number of sugar sets. The flexibility of the method should, however, be emphasised and it is obviously both undesirable and impracticable that a rigid technique should be followed in every

TABLE II

A suggested method for the investigation of pure cultures on screening plates

Reactions on plate		Presumptive diagnosis	Suggested procedure
Strains showing spreading, lactose fermentation, sucrose fermentation or pigment production		Non-pathogenic organisms	Eliminate from further investigation
Acid and gas from mannitol	H₂S+	Presumptive Salmonella	Slide-agglutinate and inoculate full set of sugars
	H₂S−	Probably paracolon bacilli but possibly *Sh. newcastle* or certain Salmonella strains	Rapid indole test. If negative after 1 hour, inoculate full set of sugars and if necessary slide-agglutinate
Acid only from mannitol	H₂S+ H₂S−	Presumptive *S. typhi* Presumptive Shigella	Slide agglutinate and inoculate full set of sugars
Non-spreading non-mannitol-fermenting strains and weak mannitol fermenters suspected of being *P. rettgeri*		Non-spreading Proteus strains, Achromobacter etc., but possibly non-mannitol - fermenting dysentery bacilli	Rapid urea test. If negative after one hour, inoculate full set of sugars and if necessary slide-agglutinate

case. Certain procedures can be added or omitted at any stage of the investigation according to the history of the case and the information required by the bacteriologist.

SUMMARY

The combined use of a screening plate, a rapid urea test and a rapid indole test in the routine bacteriological examination of fæces is described.

The method has been employed in the investigation of over a thousand colonies and has proved of particular value in the rapid recognition of presumptive pathogens and the early elimination of non-pathogens.

The advantages of picking colonies on to a single solid medium in the early stages of investigation are discussed, while the flexibility available in the later stages is emphasised.

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CRANIOPHARYNGIOMA OR PARA-PITUITARY ADAMANTINOMA (ERDHEIM'S TUMOUR)

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(PLATES LXXXI AND LXXXII)

THE case of para-pituitary tumour here reported is remarkable in three respects. First, it is unusual for a tumour of this type to attain so large a size without undergoing more extensive degenerative change. Second, the patient's symptoms dated from the termination of a pregnancy, a physiological condition which, it would seem, is unlikely to occur in the presence of such a lesion. Third, there was constant glycosuria, accompanied by an abnormal sugar tolerance curve without hyperglycæmia—an association not previously observed in such cases, although its dependence upon the cerebral lesion is more than likely.

In view of the scanty reference in British literature to this type of tumour since the study of Beckmann and Kubie in 1929, a brief review of the literature is included in the discussion.

CASE REPORT

Clinical history

First admission. A married woman, then aged 26, was admitted to the gynæcological ward, Dundee Royal Infirmary, in October 1946, complaining of amenorrhœa dating from a normal delivery in April 1939. In 1940 she had consulted her doctor and received tablets which she took regularly for about a year; during this time she apparently had regular and normal periods. When the tablets (presumably stilbœstrol) were stopped, menstruation ceased and had not returned. There was no vaginal discharge and no pain. She described a slight feeling of tension in the breasts coming on almost every month, with a little secretion lasting one day. There were no urinary symptoms. She complained of frontal headaches of some severity once or twice weekly, and of increasing irritability in the last few years. Until the middle of 1942 she had lost weight, but had regained and more than maintained her normal weight since then.

General physical examination revealed no abnormality except that the breasts were rather small, with small and retracted nipples, and the external genitals were atrophic in appearance. Dilatation and curettage were performed (Dr Buchan): the vaginal wall was atrophic and the uterus very small— 1½ inches in length, including the cervix, which was one inch in length. No endometrium was obtained. On the possibility of a post-partum pituitary

atrophy, the skull was X-rayed ; Dr T. Sprunt reported that no abnormality was present in the sella turcica apart from some interclinoid calcification. A sugar tolerance test with 50 g. of glucose was carried out. The fasting blood sugar was normal, and the curve showed a slow rise to 160 mg. and a slow and prolonged fall, being still 132 mg. per 100 c.c. after 2½ hours. Glycosuria was present throughout, being most marked after 1 hour.

Second admission. Eleven months later, on 1st September 1947, she was readmitted, this time to a medical ward, in a drowsy but rational state. The recurrent headaches which had been present for several years had, six days before admission, become extremely severe and generalised ; there was also severe pain in the neck and, on the day before admission, she had some bouts of vomiting. Since her previous discharge, there had been no return of menstruation. Latterly, she had experienced occasional difficulty in seeing objects, frequent fainting fits and unaccustomed drowsiness, and she now had to pass urine at least once nightly.

A catheter specimen of urine contained traces of albumin and sugar. The deep reflexes were exaggerated in the upper limbs but normal and equal in the lower limbs ; the plantar responses were flexor. The abdominal reflexes were present at first and later absent. The pupils were equal and moderately dilated, with sluggish reactions ; the optic discs were flat and showed marked general pallor. Lumbar puncture produced a clear fluid under increased pressure, with 8 cells per c.mm.

At 12.30 a.m. on 2nd September the patient began to have generalised extensor muscular spasms, with clenched jaw, spasticity of arms and legs and opisthotonos, but no neck rigidity. Between spasms her limbs were quite flaccid. Her temperature rose to 102·6° F. and she died 5½ hours later.

Post-mortem examination

The body was that of a woman of apparent age about 20 (actual age 27). She was of short stature and well-nourished but not obese. The head and neck were deeply cyanosed. The quantity and distribution of the pubic and axillary hair seemed normal. The nipples were retracted.

With the exception of the organs described below, and of the heart, which was small, weighing only 6½ oz., the internal organs showed nothing noteworthy.

Uterus and ovaries. The entire uterus was less than two inches in length and its cavity about half-an-inch. The endometrium was thin. The ovaries were small, fibrous and reddish on section. Histologically the uterus showed atrophy of all its elements, the endometrium being of the senile type. The ovaries had a vascular fibrous stroma containing small cystic spaces, some degenerated primordial and atretic follicles, a few corpora albicantes and no corpora lutea.

The *brain* was bulky and bulged when the dura was incised. There was moderate venous congestion of the arachnoid and extreme flattening of the convolutions. At the base of the brain a polypoidal cystic growth was found, protruding downwards for about ¾ of an inch from the region between hypothalamus and midbrain, above and posterior to the optic chiasma and immediately above the sella turcica. The chiasma was stretched and congested and the

PARA PITUITARY ADAMANTINOMA

FIG 1.—Hemisection of brain, showing parapituitary tumour mass. The upper frankly cystic portion is a polypoidal upgrowth reaching to the roof of and distending the third ventricle. Projecting forwards and somewhat downwards is the other outgrowth, showing a more solid structure, this portion had pressed down on the pituitary.

infundibulum greatly widened and continuous with the neoplastic outgrowth. The diaphragma sellæ and pituitary were compressed from above downwards, the superior surface of the anterior pituitary being concave and boatshaped. The clinoid processes were normal. A longitudinal section of the brain (fig. 1) showed the floor and almost the entire cavity of the third ventricle to be occupied by a large, pedunculated, polypoidal and cystic upgrowth of the tumour, 2½ inches in length, which bulged beyond the confines of the third ventricle after section. The tumour tissue was firm and fibrous, and the numerous small cystic spaces contained a glairy, brown, gelatinous fluid.

Histology

The *tumour* is found to be a pure type of adamantinoma or amelo-blastoma as described by numerous authors since 1904, when Erdheim showed that these para-hypophyseal epithelial tumours arise from remnants of the fœtal craniopharyngeal canal at its sites of attachment. The structure is that of a plexiform epithelioma closely resembling the adamantinoma of the jaw. Irregularly branching and anastomosing sheets or islets of epithelium lie in a delicate and sparsely cellular con-nective tissue stroma (fig. 2). The epithelial islets consist of two or three distinct zones. The peripheral zone is generally formed by a single layer of tall columnar epithelial cells of strictly rectangular outline, with large, dark-staining, oval or elongated nuclei (fig. 3), conforming in this respect with Critchley and Ironside's (1926) description of the ameloblast layer as found in the embryonic enamel organ. Some islets have a well-developed stratum intermedium of closely packed or rounded polygonal cells, while in others the ameloblast layer is contiguous with the central zone. This, in the interior of the islets, consists of a syncytium-like reticular tissue composed of round, oval, polyhedral or spindle-shaped cells with dark-staining round or oval nuclei, often eccentrically situated. In many places these cells are closely packed, with formation of numerous squamous epithelial whorls or " pearls," without, however, any keratinisation ; elsewhere the structure is looser and syncytial, with intercellular protoplasmic bridges of varying thickness, in parts resembling those of the prickle-cell layer of the epidermis. This is the " stellate " layer described by Erdheim and others, and has been likened to the reticular arrangement in the interior of the developing enamel organ. Within this central portion numerous cystic spaces, generally of small size, have formed, apparently from hydropic degeneration of the component cells ; of the other described types of cyst—the colloid and the ameloblast inclusion cysts (Critchley and Ironside)—some approximation to the former is seen, but there are no crystal-containing cysts. Despite the radiological findings during life, there is no sign of calcification in any of the portions examined histologically, and an X-ray examination of the remainder of the specimen fails to reveal it. Sections from the

floor of the third ventricle and from the infundibular region show obvious invasion of the nervous tissue, with a distinct glial reaction around the neoplastic masses.

The *pituitary* appears histologically normal, except for great flattening of the cells from above downwards.

DISCUSSION

The clinical picture in any given case of suprasellar tumour depends on a balance of effects. Nearly all the effects can, so far as we know, be attributed to simple mechanical pressure on various parts of the soft tissues within the skull, or, in the case of the hypothalamus, as in the present instance, by actual invasion of its substance by the tumour. The protean character of the syndrome from case to case is due to the juxtaposition, in the region where these tumours grow, of tissues with important and functionally diverse activities. Some of the effects, such as visual field defects, external ocular palsies and optic atrophy, are purely neural and are relatively easily recognised as such. Others are endocrinal, and manifest themselves only by the resulting secondary upset of the general endocrine balance; it may therefore be impossible, as in the metabolic derangement of the present case, to disentangle the primary abnormalities with any accuracy. Modern knowledge allows us to say that certain signs and symptoms are specific evidence of dysfunction of the optic chiasma, while other signs point with equal certainty to the pituitary or hypothalamus, but overlying these, and possibly altering the balance between such regionally determined factors, there can be the general effort of increased intracranial pressure, whether by mere growth of the tumour or by varied degrees of blockage to the flow of cerebrospinal fluid. The varied syndromes thus encountered are available in the scattered case reports and reviews, especially those of Critchley and Ironside (1926), Beckmann and Kubie (1929), Frazier and Alpers (1931), Cushing (1932) and Friedgood (1946). The amenorrhœa, polyuria, abnormal drowsiness and optic atrophy of the present case are all of common occurrence in adult patients. The terminal tonic muscular spasms and pyrexia are less common but have been reported by Critchley and Ironside and Beckmann and Kubie; it is of interest that the former also record spastic attacks following operation, and suggest a sudden hypercholesterolæmia as the explanation.

In the present case the original onset of symptoms followed parturition and this, with the subsequent general atrophy, suggested at that time a clinical diagnosis of Simmonds's disease; it was not then known that this syndrome may be due to a suprasellar tumour (Summers, 1947). No other example of a tumour of this type in a parous woman has been found in a review of 204 recorded cases. One of the cases described by Frazier and Alpers, a married woman of fifty, had miscarried once. They comment on the rarity of marriage

PARA-PITUITARY ADAMANTINOMA
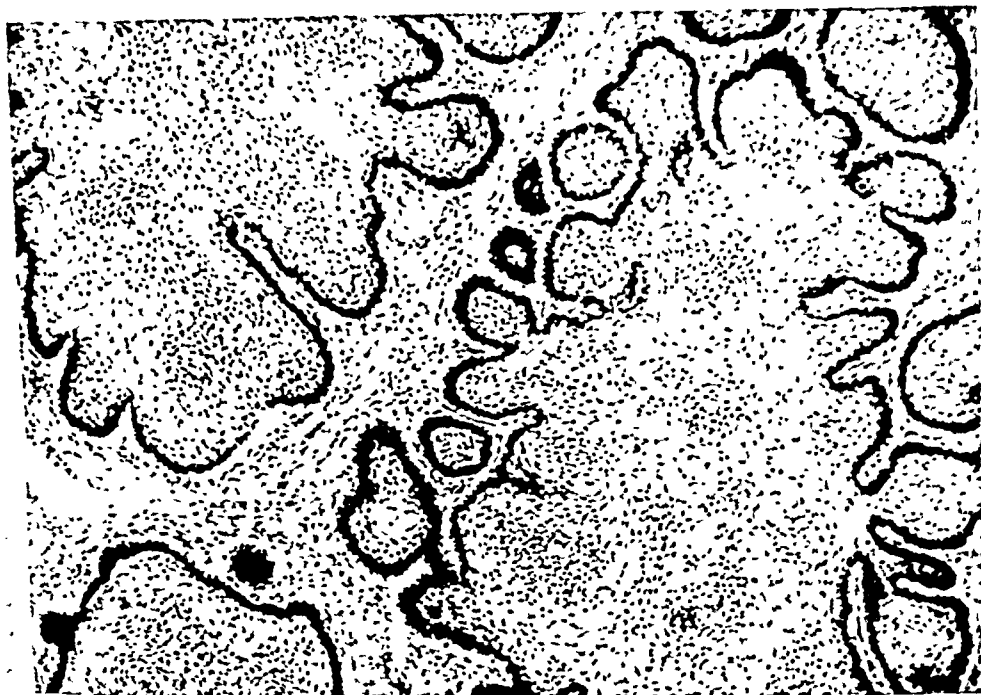
FIG. 2.—This shows the characteristic epithelial masses with their club-like projections, clearly outlined by the crowded nuclei of the ameloblast layer. Internally the epithelial mass is frayed out into an œdematous or myxoid reticular arrangement, with foci of closely aggregated whorled cells. It is degeneration of this central epithelial zone which produces the commonly occurring cysts. Celestin blue, hæmalum and Van Gieson. × 70.
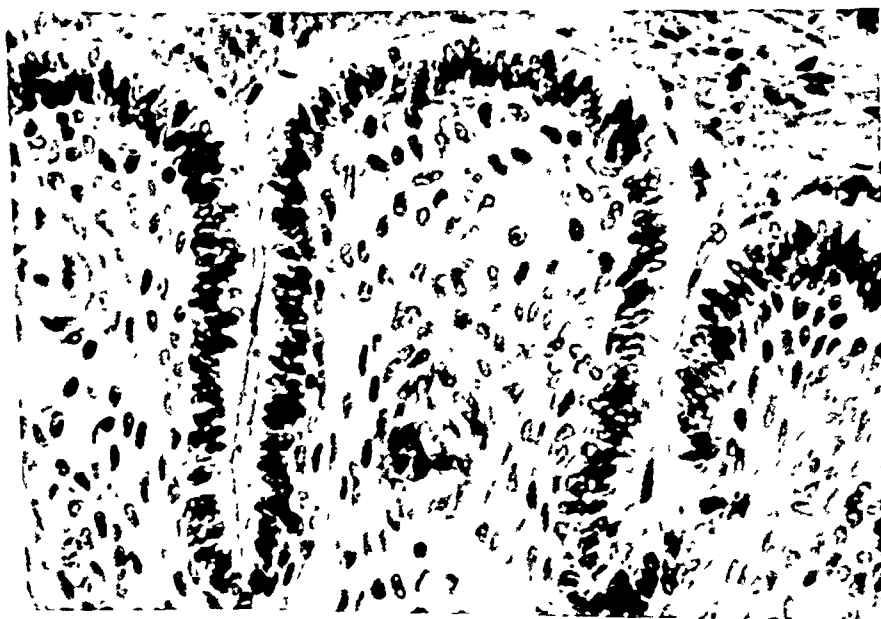
FIG. 3.—The periphery of the epithelial mass, showing the heaped-up layer of nuclei of the so-called ameloblasts, and the fact that they are all confined to the near end of the cells. There is some retraction from the adjoining stroma above, an artefact which helps to reveal the delicate membrane at the peripheral (clear) end of the cells. One of the characteristic non-keratinised whorls is present in the middle club. Celestin blue, hæmalum and Van Gieson. × 340.

in female subjects. Indeed, none of the eight adult female patients of Beckmann and Kubie had married, and they suggest that women with this tumour have an early disturbance of the psychosexual constitution.

Various upsets in the metabolism of sugar have been recorded in this disease. Glycosuria, when present, has always been associated with hyperglycæmia, but in the present case sugar appeared in the urine when the blood sugar was as low as 90 mg. per 100 c.c. The sugar tolerance curve revealed a peculiarly slow rise to a maximum of only 160 mg., with a subsequent very slow fall. The flatness of the curve seems to be fairly characteristic; it was found in Conley's (1941) case and two of Critchley and Ironside's cases, while Beckmann and Kubie describe it in five cases, two of whom, nevertheless, had hyperglycæmia and glycosuria. The explanation of this flat curve is not easy. Although on the one hand it is well known that lesions in the hypothalamic region may cause hyperglycæmia and glycosuria by altering the sugar storage capacity of the liver, on the other, a lessened activity of the pituitary may be characterised by hypoglycæmia (Friedgood). As both hypothalamic damage and pituitary hypofunction are likely to be present in a case of craniopharyngioma, it is not inconceivable that the abnormal sugar tolerance frequently found in this condition may be a mixture of these two effects. The peak figure in most of the recorded sugar tolerance curves has been low, and it was only in the exceptional cases showing a high plateau that glycosuria was recorded. It may be that the low renal threshold for sugar in the present case is a chance association; none the less, despite its absence in the other reported cases, it is tempting to regard it as yet another expression of the multiglandular syndrome resulting from damage to the pituitary and hypothalamus.

Since Erdheim's original and clarifying paper on hypophysial duct tumours in 1904 and his later review in 1925, a considerable literature has appeared, mainly in America. The ingenious hypothesis put forward by Erdheim, that the tumours are derived from squamous epithelial rests of the embryonic craniopharyngeal canal, has been generally accepted and elaborated in subsequent studies, and it is apparently supported both by the embryologists' observations and by the frequency with which squamous epithelial rests have been found in the normal hypophysis (Carmichael, 1931). More recently, however, this view has been challenged by Tilney (1936), who follows Atwell (1918) in declaring that there is no good proof that any part of the canal persists in the adult state in man; he traces the origin of the tumours rather to inclusions in the pars tuberalis of elements from the embryonic dental plate. Embryologically, this view seems not to be so very different from that of Erdheim, and in any case, as Willis (1948) remarks (p. 628), the resemblance between adamantinoma of the jaw and many stalk tumours "is not surprising, considering that both sets of residues have a closely similar develop-

mental origin and consist of closely similar foci of indifferent epithelium.''

An entirely different approach to the pathogenesis is suggested by Weil and Blumklotz (1943), who produced three epithelial cysts at the base of the brain in rats by the implantation of methylcholanthrene crystals. One of these growths is stated to have shown a distinctly adamantinomatous appearance, while the other two were similar to squamous papillary cysts; they attribute the cysts to neoplastic transformation of the arachnoidal mesothelial lining, and appear to regard their views on the histogenesis as satisfactorily replacing the orthodox theory. Their findings, however, are by no means clearly related to the tumours under study. In brief, the view of the pathogenesis generally held to-day is still that of Erdheim, namely that certain tumours arise from squamous epithelial rests in the line of the embryonic craniopharyngeal canal, especially in the upper part of the infundibulum—the commonest site—and between the lower end of the infundibulum and the anterior lobe of the pituitary; hence the tumours may be either suprasellar or intrasellar in position, the former being much the commoner. The greatest number of those reported have been in relation to the suprasellar or upper part of the infundibulum, the others have lain in the pituitary itself or below it.

The tumours included in the Erdheim group are not quite uniform in type. The adamantinomatous variety is the most frequent, but differences in histological structure of considerable degree have been noted, both from case to case and even within the same tumour. There is likewise difference in behaviour from case to case. It is thus rather difficult to produce a satisfactory classification of the tumours taking origin from the embryonic craniopharyngeal canal (Duffy, 1920; Frazier and Alpers, 1931; Friedgood, 1946) and it is obvious that with increasing knowledge the suggested categories are having to be modified and subdivided. What may be called the typical para-pituitary adamantinoma or basal-cell carcinoma, of which the present case is a good example, is only locally invasive; it is usually solid, although multiple cysts may be present, especially if the tumour itself be large. The next most common type would appear to be the monolocular cyst with a papillary squamous lining. This, like the next variant, the solid squamous growth, is a benign tumour. frequently showing obvious transitions to the usual adamantinomatous picture. Where the monolocular cyst is characterised by excessive keratin formation with crystallisation of cholesterol, some writers have preferred the term cholesteatoma, but such sterol-containing cysts occur in any of the variants already described and the use of this term would be better dropped. The possible occurrence of a frank squamous carcinoma in this region has been repeatedly affirmed, but it must be a rare event. All these tumours can reasonably be taken as forming a single group, derived from Erdheim's squamous epithelial rests.

There remain two types of tumour in this region which in the past have been confused with the Erdheim rest tumours (cranio-pharyngioma). These are the so-called cysts of Rathke's pouch and the teratomata. Monolocular cystic tumours with cylindrical, stratified or ciliated epithelium are included, under the name of " true Rathke pouch tumours ", along with the adamantinomata in Frazier and Alper's classification of neoplasms arising from the embryonic craniopharyngeal canal. It is obvious, however, from the work of Baar (1947), that these cysts are not true neoplasms and have quite a different origin from the Erdheim rest tumours. They are really colloid-containing retention cysts resulting from failure of Rathke's cleft to divide into the small colloid cysts normally found in the pars intermedia of the pituitary. These monolocular Rathke cysts are generally lined by cubical epithelium, but sometimes the lining is of ciliated epithelium and it has been suggested that its presence is due to the onset of the abnormal development at an earlier stage in fœtal life (Baar). Neither type is neoplastic and neither has histogenetically anything in common with the Erdheim tumours. They are, moreover, always intrasellar in situation. As for the teratomata, they are included in Frazier and Alper's classification as " probably " occurring. It is known now that teratomata undoubtedly occur in this situation and that they have no relation to the tumours under discussion, except the chance one of situation. In the interesting series of fourteen supra-sellar tumours, most of them described as squamous papillary cysts, published by Globus and Gang (1945-46), cartilage, bone and acinar structures were each found in one, hair follicles in two, and sebaceous cells in two ; and although these writers do not emphasise the teratomatous character of these tumours, and presumably regard the bone as metaplastic as sometimes happens (Erdheim), it is just possible that they were, in fact, describing teratomata.

The vital importance of early diagnosis in cases of suprasellar tumour has been stressed repeatedly, but the only clues, as the present case shows, may be not only slender, but almost misleading. When the lesion declares itself before adolescence, the results of endocrine disorder, such as obvious stunting, sexual immaturity and adiposity, and also the symptoms of increased intracranial pressure, are usually obvious at an early stage, whereas in adults the tumour may grow for years without obviously pointing to the pituitary. Neglect or misinterpretation of an early sign may, through delay, make hopeless a possible case for surgical removal. One of the few relatively constant early signs is amenorrhœa, and it seems clear now that, in the absence of any other explanation, this symptom should suggest the possibility of suprasellar tumour. Confirmation should be sought by radiography of the skull for suprasellar calcification, by a glucose tolerance test, and by examination of the ocular fundi and visual fields.

SUMMARY

A case of craniopharyngioma (para-pituitary adamantinoma) in the suprasellar position is described. The patient was a young parous woman, a circumstance which is rare if not unique. The symptoms, at first merely amenorrhœa, followed immediately after the pregnancy and suggested post-partum pituitary atrophy.

There was glycosuria, which is uncommon, unaccompanied by hyperglycæmia; this has not been previously recorded.

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NEPHROBLASTOMA OCCURRING IN ADULT LIFE

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(Plates LXXXIII-LXXXV)

Some of the earliest accounts of " embryonic " or " mixed " tumours of the kidney refer to examples in adults, and additional cases of this kind have been reported in the literature from time to time. Among these, a few are clearly authentic examples of " mixed " renal tumours, others, just as clearly, are not. The greatest obstacle to any understanding of the exact nature of most of these tumours— and this applies to both the older and the more recent accounts—is the absence of adequate objective information in the descriptions and illustrations. Hence, although the existence of occasional adult tumours of this type cannot be questioned, no adequate idea can be obtained of their frequency, or even of their characteristics as a group. For this reason the present tumour, appearing in an adult but with a structure closely resembling the nephroblastoma of childhood, is described in detail.

CASE REPORT

Clinical history

The patient, a man 31 years of age, sought medical advice because of an enlarging abdominal tumour which had been present for some months. His first symptom had been a transient attack of hæmaturia two years previously, but he had had no medical attention at that time. Clinical investigation revealed a bulky tumour of the right kidney, an enlarged and irregularly-contoured liver and a mass in the region of the right groin. No treatment was attempted, and the patient died two months after admission to hospital.

Autopsy findings

On incision of the anterior abdominal wall the viscera were seen to be displaced by an ovoid tumour 14 in. in length and 10 in. in breadth, replacing the right kidney. The right dome of the diaphragm was displaced by the tumour to a position about 4 in. above its normal level, while the liver, spleen and stomach were pushed over to the extreme left of the upper abdomen. To the right of the mid-line the tumour was adherent to the anterior abdominal wall. The ascending

and transverse portions of the colon were stretched tightly across the lower part of the tumour and were incorporated in a thick outer fibrous capsule, formed by the mesentery and omentum, to which the tumour itself was firmly adherent. The small intestine and descending colon were confined to the pelvis and lowest part of the left side of the abdomen. Above the upper part of the tumour, beneath the dome of the diaphragm, the normal right adrenal was identified in relation to the displaced liver.

When removed from the body, the tumour, which weighed 14 lb., showed a ragged contour where it had been adherent to adjacent viscera, but on its superior and medial aspects it was covered by a smooth capsule. On its posterior aspect near the original site of the hilum an outer shell of thinned-out renal tissue was preserved, but over the rest of the surface no such tissue could be identified. When the specimen was cut, the well-preserved peripheral parts of the tumour were found to be soft, but uniformly white in colour. Centrally it had undergone extensive degeneration, with formation of many ragged cavities lined by disintegrating brownish tissue and containing dark fluid.

There was no gross invasion by the tumour of the renal vein or its major tributaries, nor were any deposits present in abdominal or thoracic lymph nodes. The liver and lungs contained many round white metastatic tumours ranging up to 2 in. in diameter.

Examination of the mass in the right groin showed it to be a deposit of growth in the superior ramus of the pubis, expanding this bone to a smoothly-contoured mass 4 in. in diameter.

The rest of an exhaustive autopsy revealed no further significant abnormalities, and the anatomical diagnosis was carcinoma of the right kidney, with metastases in lungs, liver and bone marrow.

Pathological histology

In the primary renal tumour and in all its metastases, the general structure is the same. Even at a first glance the lesions present an appearance quite unlike that of an ordinary renal carcinoma of adult type. The bulk of the tumour tissue has the type of structure illustrated in fig. 1, and consists of an aggregation of rounded or elongated glandular structures. The nuclei of the cells making up these tubules are round or spindle-shaped and darkly staining ; mitoses are frequent. Only traces of cell outlines can be identified, usually in the inner parts of the glandular formations, the nuclei being basally situated. Shrinkage in preparation has caused some separation of the glandular structures, in places bringing into prominence a thin layer of delicate fibrous stroma between the tubules. Where a recognisable inter-tubular stroma is present it is continuous with the fibrous tissue of the organ containing the tumour, or with the surrounding fibrous capsule where one is present. Like the stroma of a purely epithelial

PLATE LXXXIII

NEPHROBLASTOMA IN ADULTS

FIG. 1.—Peripheral part of a pulmonary metastasis, showing an area of tumour tissue which consists of glandular formations with a small amount of intervening collagenous stroma. ×60.
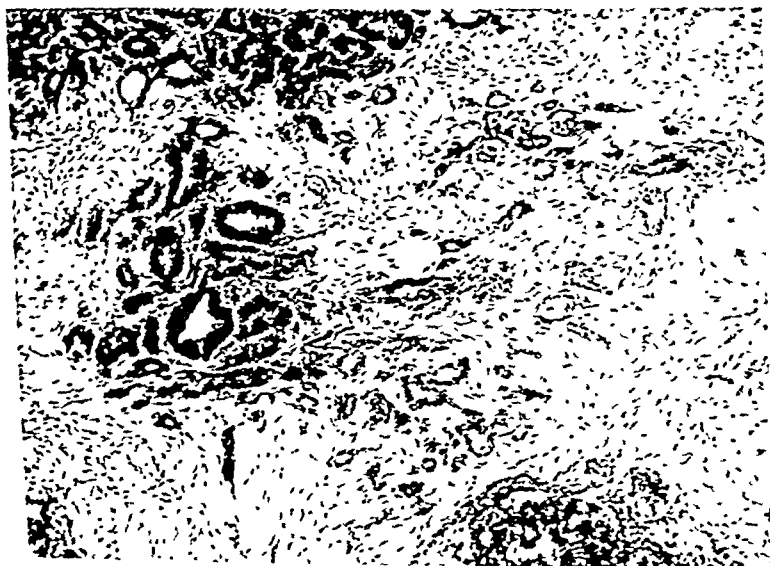
FIG. 2.—The more central part of the same pulmonary metastasis, showing an area of tumour tissue where the fibrous stroma is very prominent. ×100.

tumour, it appears to have developed from the local supporting tissue of the part.

An area of the tumour in which this stroma is very prominent is shown in fig. 2, where clearly recognisable glandular formations of tumour cells are seen, surrounded by vascular connective tissue. Although in close contact, the two components are quite distinct.

In other areas of the tumour there exists a more intimate structural relationship between the cells of the epithelial formations and those surrounding them. It is this relationship which enables the present lesion to be grouped with the " embryonal " renal tumours. In these situations the proliferating masses of tumour tissue consist not only of recognisably epithelial cells, but of a mixture of these with interlacing strands and bundles of spindle-shaped cells. Occasionally the spindle-celled tissue predominates, in some areas to such an extent that the tumour has the appearance of a sarcoma. More commonly, glandular formations are scattered throughout the apparently sarcomatous tissue. From figs. 3-5, selected from such areas in separate pulmonary metastases, it is evident that there are numerous transitions between the epithelial cells and their spindle-shaped neighbours, and that the glandular structures and spindle-celled tissue represent modifications of a common cell type. The complex cell aggregates so formed are exactly comparable with those of the ordinary nephroblastomas of childhood.

The complexity of structure and inter-relationships of the histological pattern can best be seen in the earliest stages of growth of the pulmonary metastases. The metastatic deposit, when it can be recognised at a sufficiently early stage, is seen to consists of a mass of undifferentiated spindle-celled tissue. A fragment of this kind impacted in a small pulmonary artery is seen in fig. 6. A mass of recently formed thrombus lies alongside the tumour embolus, and there is as yet no invasion of the vessel wall. In the vessel shown in fig. 7, progressive development of a similar embolus has resulted in the establishment of a recognisably glandular pattern amongst the tumour cells. The entire lumen of the blood-vessel is occupied by the mass of growth, but although this is everywhere adherent to the wall of the vessel, no extension to surrounding tissues has occurred, nor has tumour tissue as yet become vascularised by the ingrowth of capillaries.

Fig. 8, showing a larger and more fully established metastasis, illustrates stromal development at an early stage. The tumour is still confined to the lumen of the artery, but growth of the embolus has so expanded the vessel and stretched out its wall that the muscle coat is barely recognisable. The tumour tissue, originally spindle-celled, has produced numerous peripherally situated glandular structures, while the centre is occupied by a vascular connective tissue stroma which has grown into the tumour from the vessel wall. A small residual portion of thrombus is situated at one margin of the tumour.

No smooth or striated muscle fibres, cartilage or bone have been found in the tumour in spite of prolonged search.

DISCUSSION

The earliest accounts of the tumour now known as a nephroblastoma (those of Eberth, 1872; Cohnheim, 1875; Birch-Hirschfeld, 1898; Muus, 1899; Wilms, 1899; and Hedrén, 1907), which laid the foundations for current ideas on their pathology and histogenesis, appeared at a time when knowledge of other types of renal tumour was uncertain and confused. Stoerk's (1908) refutation of the adrenal origin of " hypernephroma " had not yet appeared, and the renal tumours of adults were regarded as a diverse and heterogeneous group. It is not surprising, therefore, that as knowledge of the structurally complex renal tumours of childhood became familiar, there were selected from this apparently confused assortment of adult renal tumours examples which, because of certain supposedly unusual features, were classified with the childhood group.

The first of these cases, described by Hoisholt in 1886, appears to have been a genuine " mixed " tumour. It occurred in a youth of 18, who at autopsy presented a massive renal tumour with metastases throughout the peritoneum and in the right lung. The tumour is described histologically as a sarcoma, with areas of cartilage and local collections of spindle cells which were thought to be non-striated muscle fibres. No striated muscle could be identified and no mention is made of any epithelial component.

Another apparently authentic example is the case described by Muus (case III). It was that of a woman aged 24 years who died with a huge renal tumour 14 days after the birth of a child. The tumour had a structure which the author declared to be typical of the " mixed " renal tumours of childhood, and in support of this contention he described spindle-celled tissue with multiplying cells arranged in local aggregates whose central parts contained · tubular structures. Striated muscle fibres and fat cells were also said to be present.

A third plausible example from this early period is that of Jenckel (1901)—a bulky tumour in a woman aged 43 years. This is described as consisting of connective tissue and epithelial elements. These took the form of "nodes" of epithelial cells, with glandular formations which communicated with cysts lined ·by cubical epithelium. Non-striated muscle and fat were present among the connective tissue elements, but neither striated muscle fibres nor cartilaginous islets were discovered.

In the absence of illustrations none of these examples can be· considered as fully established, but the descriptions are sufficiently detailed to carry conviction, and what is described conforms to the general pattern seen in nephroblastoma. Other early accounts are not so easily interpreted, because they are often described in the confused terminology of the time and lack adequate microscopic illustrations. Nevertheless, in the light of more recent experience ˂ of the range of structural variation exhibitied by adult renal carcinomas, a present-day reader has no difficulty in deciding that some of the tumours described were merely ordinary renal carcinomas which,

NEPHROBLASTOMA IN ADULTS

FIG. 3. — Pulmonary metastasis showing aggregates of epithelial cells and a clearly recognisable tubular structure in the midst of spindle-celled tumour tissue. ×180.

FIG. 4.—Pulmonary metastasis. Another area of complex structure showing how the cells of the glandular formations merge with the adjacent spindle-celled tissue. ×180.
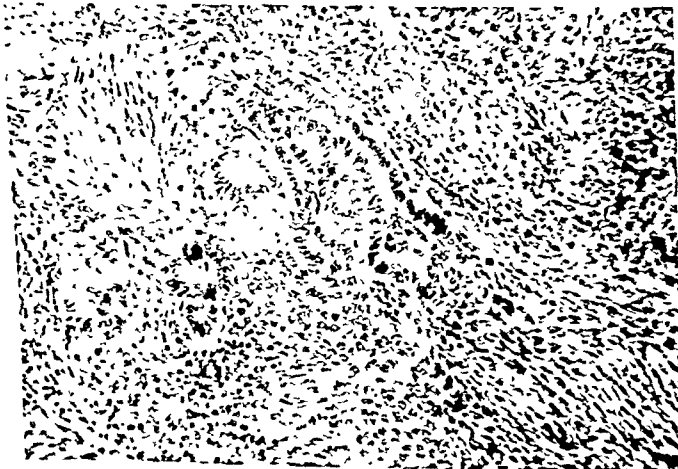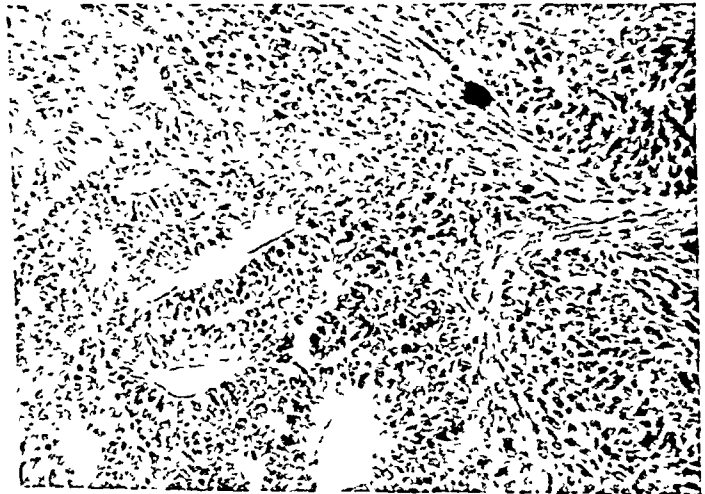
FIG. 5. — Pulmonary metastasis. Another field showing the close relationship between the cells of the glandular structures and those of the intervening spindle-celled tumour tissue. ×150.

because they presented occasional atypical areas of anaplastic un-
differentiated tissue, eluded correct identification. Cases of this sort
include those of Lotheissen (1896, case 4)—a woman of 38 with a
tumour the gross and microscopic descriptions of which appear typical
of ordinary renal carcinoma ; of Hedrén (1907, case 4)—a woman
of 54, areas of whose tumour are stated to have the usual structure
of a renal carcinoma ; and of Nicholson (1909)—a woman of 40,
whose tumour also is stated to contain areas identical in structure
with " hypernephroma."

The following tumours are of greater interest. The first, described by
Reid (1896), was in a woman of 28. It was a bulky tumour, described as a
sarcoma which showed tubular structures and indistinctly striated cells in the
spindle-celled tumour tissue. The next, that of Blau (1898, cited by Hedrén) in
a man of 22, again a bulky tumour, had produced metastases in the liver and
lungs. It contained numerous longitudinally and transversely striated cells and
was described as a " myosarcoma striocellulare." The third, that of Busse (1899)
in a woman of 57, was described as a rhabdomyoma of the renal capsule. These
three tumours may well belong to the nephroblastoma group but on critical
examination this diagnosis cannot be regarded as substantiated.

Cases where the nature of the lesion must be regarded as quite uncertain
are those of Wyss (1901)—a woman of 37 who had a cystic spindle-celled
bone-containing tumour ; and of Keefe and Palmer (1910).

The fashion of diagnosing adult nephroblastomas still persists, as does also
the habit of omitting from the published reports the objective information or
microscopic evidence on which such a diagnosis may be based. As before,
from what little evidence is available, many of the tumours, it may be surmised,
are ordinary adult renal carcinomas. This applies to the cases of Kilbane and
Lester (1929), Thatcher and Fulmer (1929), Busser *et al.* (1938), Hultquist (1938),
Papin *et al.* (1938), Hamm (1942), Sparks (1942), Moore (1942-43), Wood (1944)
and Oesterlin (1945). No conclusions at all can be reached concerning the
cases of Rohde (1919), Derrick (1922), Baumann (1923), Davis (1931), MacDonald
(1927-28), Jenkins (1931), Neff (1931), Dean and Pack (1932), Geschickter and
Widenhorn (1934), Priestley and Broders * (1935), Alcorn (1937), Nelson (1939),
Livermore (1943), Loeb (1943), Bell (1946), Hill (1946) and Twinem (1946).
The diagnosis cannot be said to have been substantiated in any of these ; indeed
in this more recent period the only example to have been established with any
degree of certainty is that of Hasner (1928). This tumour, in a man of 45, is
described as being composed of tubules and embryonal striated muscle cells.
An adequate photomicrograph confirms the presence of these striated cells
and establishes the lesion as an authentic " mixed " tumour.

A case quoted by Derrick (1922) as an example of adult nephroblastoma
is that of Wohl (1917), who himself made no mention of its relationship to the
embryonal group. The tumour, which occurred in a man of 31, was described
as a malignant papillary adenoma of the kidney. From Wohl's account it is
clear that this term is not applicable in its usual sense, and as the histological
illustrations show a close resemblance to the glandular structures of the tumour
described in the present paper, it is just possible, as Derrick suggests, that the
tumour was a nephroblastoma.

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* Dr Broders was kind enough to send me sections of the two tumours listed (but
not described) in this paper as adult nephroblastomas. They were frozen sections of
material that had been stored for many years, but they show the tumours to be
unusual ones. Certainly in one case there are adequate grounds for the diagnosis of
nephroblastoma.

One other lesion to be considered here is that recorded by Marshall (1943). His description refers to a surgically resected kidney from a woman of 40 which contained a pale grey encapsulated cystic and hæmorrhagic mass 13 cm. in diameter. From the histological structure the possibility of its being a metastatic tumour is discussed and rejected, and it is judged to be normal endometrial tissue, composed of epithelial tubules embedded in a spindle-celled stroma containing some smooth muscle. In view of the rarity of a lesion of this nature in the tissues of the kidney, it is suggested, as a possibility, that this may be an example of nephroblastoma in an adult.

Because of the many uncertainties revealed in a critical survey of the cases described or quoted as examples of nephroblastoma in adults, it is suggested that in future all observed adult examples of this type of tumour should be recorded, and that adequate objective evidence of their structural characteristics should be included in the published report.

SUMMARY

A renal tumour of unusual structure occurring in a man 31 years of age is described. It bore no histological resemblance to the usual type of renal carcinoma, but, on the contrary, was similar to the group of " mixed " tumours of the kidney that occur in childhood. It consisted of a mixture of epithelial tubules and spindle-celled tissue, these intimately related components representing modifications of a common cell type.

Cases described or quoted in the literature as adult examples of this type of renal tumour are reviewed. In the great majority the diagnosis is in doubt, and in many it is apparent that the tumour was an adult renal carcinoma. Lack of familiarity with the wide range of structural variation exhibited by tumours of this group appears to be the usual cause of misdiagnosis.

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FIG. 6.—Section through a small (250 μ diameter) pulmonary artery containing an arrested embolus of spindle-shaped tumour cells. × 150.

FIG. 7.—Section through another small (200 μ diameter) pulmonary artery. The lumen of the vessel is completely occupied by a mass of tumour tissue which shows the development of a recognisably epithelial pattern. × 180.

FIG. 8.—The growth of this intra-arterial tumour has locally expanded the vessel. Glandular structures occupy the peripheral part of the growth, while the central part consists of vascular connective tissue stroma. × 60.

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EXPERIMENTAL PULMONARY ŒDEMA OF NERVOUS ORIGIN

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(PLATE LXXXVI)

WHILE engaged in an experimental study of hydrocephalus we hit upon a device which proved remarkably successful in producing acute œdema of the lungs. The method depends upon the cisternal introduction of freshly prepared fibrin mixture through the foramen magnum, whereupon the animal either dies at once through respiratory collapse or becomes dyspnœic and passes into acute pulmonary œdema within 5-10 minutes of injection. We present evidence in this paper that the underlying mechanism of the œdema is nervous in origin. One of us has briefly referred to the method in a lecture (Cameron, 1948) and we have communicated a summary of the earlier results to the Pathological Society of Great Britain and Ireland in July 1948.

METHODS

The procedure was successful with rats and rabbits and quite likely could be applied to other experimental animals, though we have had no experience with these. Under light open-ether anæsthesia for rats and intraperitoneal Nembutal anæsthesia followed by ether for rabbits, the fur was removed with clippers from the posterior surface of the neck and head, the atlanto-occipital gap defined and the fibrin mixture injected slowly into the basal cisternæ by means of a 1 or 5 c.c. syringe fitted with a short bevelled needle If the rate of injection was too rapid, the animal stopped breathing at once and seldom recovered ; occasionally the prompt application of artificial respiration would re-start natural breathing and the sequence of events follow the same course as with slow injection of the fibrin. The fibrin was prepared by mixing four parts of fibrinogen diluted 1 in 10 in sterile distilled water with one part of thrombin diluted 1 in 5 in sterile normal saline immediately before use, 0·2 ml. of the mixture being injected intracisternally into rats and 3 ml. into rabbits. We wish to thank Dr Kekwick of the Lister Institute for a generous supply of these substances.

We also injected by the same route in the case of rats fresh rat blood, heparinised and non-heparinised, and human plasma and fibrinogen and thrombin alone, normal saline and particulate suspensions such as India ink and colloidal dyes into both rats and rabbits. Doses comparable with or greater than those used for the fibrin mixture were employed, and experimental procedures were carefully standardised in all instances. Since the duration of the experiment was short, we generally took no special precautions about asepsis, although in some instances we controlled this factor and assured ourselves that it is of no

importance. Finally we injected human and rat serum, plasma, fibrinogen and thrombin intraperitoneally and intradermally into rats in order to make sure that none of these procedures induced pulmonary œdema by absorption from the tissues into the blood stream. These control experiments are necessary, for Green and Stoner (1947) have shown that thrombin injected intravenously gives respiratory phenomena not unlike those recorded by us, although they used much larger doses than ours. Our control experiments gave no evidence of the passage of thrombin into the blood after intracranial injection; moreover, the disturbances we describe developed in heparinised as well as non-heparinised animals, a point of fundamental difference between Green and Stoner's experiments and ours.

For the investigation of circulatory changes we recorded pressure variations within the right auricle and the left carotid artery concurrently by the kymographic method. Rabbits alone were used in this work. They were heparinised by the injection into an ear vein of 1 mg. heparin dissolved in 3 ml. distilled water prior to anæsthesia. The right lower external jugular vein was exposed and cleaned of all fat and its tributaries were divided between ligatures. A narrow plastic tube of 1 mm. internal and 1·8 mm. external diameter, kindly given to us by Dr Sheila Howarth, was introduced into the vein and passed towards the heart for a distance of about 6 cm., being fixed *in situ* by means of a stout thread. Previous experiments had shown that this distance ensured certain catheterisation of the right auricle. In every case we verified the position of the catheter in the auricle at the end of the experiment. The remarkable constancy of pressures so recorded from animal to animal also suggests that the method was satisfactory. The proximal end of the catheter was attached by narrow rubber tubing to a manometer containing normal saline and pressure variations were obtained by means of a light hollow ebonite float with an aluminium stylet. The left carotid artery was then canularised in the usual fashion and its pressure recorded by means of a mercury manometer, float and stylet, the rubber tubing connecting the arterial canula and the manometer being filled with normal saline. The animal was maintained in the horizontal position throughout the experiment.

Respiratory movements were recorded by winding a pneumatic cuff round the lower part of the chest, its nozzle being attached by rubber tubing containing air to a manometer filled with normal saline. A stylet connected to an electrical chronometer recording every five seconds was adjusted to the lowest level of the kymograph paper.

In some experiments, both vagus nerves were gently isolated from the posterior aspect of the carotid sheaths and divided between ligatures at the middle of the neck after the pressure recordings had been going on for some minutes and shortly before fibrin injection. When rats were used for vagotomy observations, we found that it was best to divide one vagus before cisternal injection and the other immediately after completion of the injection, since the rat tolerates simultaneous double vagotomy poorly.

For reasons which we shall give later, it was necessary to study the effects on the circulation of tracheal obstruction and pulmonary embolism. To produce obstruction, the trachea was gently dissected from its surrounding tissues and a strong thread was slipped around it and steadily tightened while vascular and respiratory pressures were recorded in the manner already described. Pulmonary embolism was produced by injecting intravenously a suspension of potato starch granules prepared by the method of Dunn (1919-20). Five ml. of this suspension sufficed to give severe obstruction of the pulmonary vessels, with death in 5-10 minutes.

At the termination of each experiment the chest contents were carefully examined, lungs and heart being removed after the trachea had been ligatured. The heart was separated and its cavities opened and cleared of blood, washed

and blotted dry, and the lung : heart weight ratio determined. In many instances histological studies of the lungs were carried out after fixation of the tissue in 10 per cent. formol-saline, paraffin embedding, and staining of sections 6 μ thick with Ehrlich's acid hæmatoxylin and eosin and Weigert's iron hæmatoxylin and Van Gieson. Œdema fluid was gently aspirated from the trachea, centrifuged, and its protein content kindly estimated for us by Dr K. K. Cheng, using the micro- method described by Rimington and Bickford (1947).

Finally we studied the distribution within the cranial cavity of the fibrin mixture by adding to it a little iron ammonium citrate and potassium ferrocyanide. After injection of this mixture the brain was removed and fixed in one per cent. hydrochloric acid in ten per cent. formol-saline, which showed up the fibrin vividly because of the formation of prussian blue. Thick paraffin sections at various levels of the hind brain enabled us to trace the course of the fibrin-prussian blue mixture throughout the ventricles and their recesses.

Details of the drugs used in our experiments are given in the appropriate sections.

RESULTS

The phenomenon

Fifteen of 18 rats injected intracisternally with 0·2 ml. fibrin-forming mixture developed acute pulmonary œdema, the remaining three dying immediately after the injection. All of a group of nine rabbits receiving 3 ml. fibrin mixture showed œdema of the lungs. The phenomenon occurred in heparinised as well as in untreated animals.

The animals showed a characteristic behaviour. Even before completion of the injection the voluntary muscles went into tonic contraction, the body became a little flexed, the hairs became erect and quivered, and the eyes bulged and the pupils dilated widely. Sometimes urine and fæces were discharged. After 1-5 minutes the body relaxed completely and profuse frothy fluid gushed from the nose and mouth just before death ; in the less serious cases this did not occur, although the lungs of such animals became distended with œdema fluid. One or two deep inspirations preceded a prolonged period of apnœa in rabbits ; rats showed a deep inspiration at once after injection, which was followed by severe and rapid gasping respirations, terminating with the outpouring of œdema fluid.

On opening the chest, the typical picture of severe acute pulmonary œdema was found, identical in its macroscopic appearance with that after gassing with phosgene. The lungs filled the chest and failed to collapse. They overlay the heart and were mottled, especially posteriorly, with dark red patches of congestion and hæmorrhage. On section, the cut surfaces poured out pink or dark red fluid. The larger air spaces were filled with a similar fluid. The edges of the lungs were often emphysematous, and the main vessels to the organ appeared to be distended with fluid blood. Dilatation of the cavities of the right heart was a common finding. The lung : heart ratio varied from 3 : 1 to 5·2 : 1 as against the normal ratio of 2·4 : 1 given by Dunn (*loc. cit.*).

Microscopical examination shows that most of the air spaces are filled with a homogeneous eosinophilic exudate rich in protein and containing a variable number of red blood corpuscles. Some alveoli may be plugged completely with these cells so that the septal capillaries are obscured; in others fluid plasma predominates and corpuscles are found with difficulty. The pulmonary blood vessels, large and small, are usually distended with blood corpuscles, but no evidence
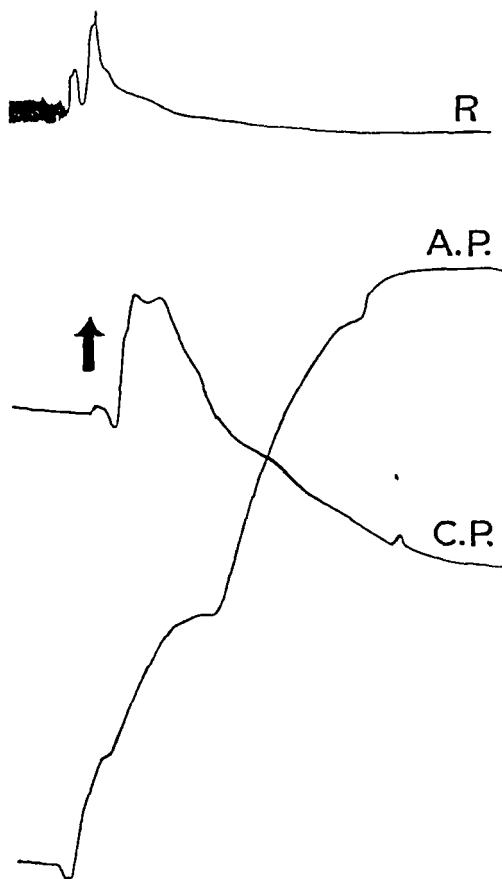
Fig. 1.—Kymographic tracing of respiration (R), carotid arterial pressure (C.P.) and right auricular pressure (A.P.) in a rabbit before and after cisternal injection of 3 c.c. fibrinogen-thrombin mixture. Arrow indicates time of injection of mixture.

of injury or breach of vessel walls is found even after prolonged search. A striking feature is the tremendous distension of the peri-bronchial and perivascular lymphatic spaces : this too is reminiscent of phosgene œdema. No doubt this structural abnormality is associated with a greatly increased flow of lymph from the lungs as has been recorded by Drinker (1945) and Cameron and Courtice (1946-47) in dogs exposed to various noxious gases. Damage to bronchial epithelium or excessive mucus production is not apparent. The protein content of œdema fluid collected from 2 rabbits and 4 rats ranged between

4·7 and 5·5 per cent. after centrifuging out the cells, figures which indicate that the fluid is almost pure plasma. It may be concluded with reason that the lung capillaries had rapidly become abnormally permeable to proteins as the result of the experimental procedure. Indeed, it looks as if some of the vessels had allowed whole blood to escape, although there is no sign of rupture of their walls.

While these changes were moving to their climax in the lungs, the circulatory system was displaying interesting reactions. The carotid arterial pressure at once increased from the normal range of 80-120 mm. Hg. by 20-50 mm. and usually persisted at this high level for a variable time, falling gradually to subnormal levels and eventually to zero at death (fig. 1). In two rabbits, however, arterial pressure fell by 5-8 mm. Hg. for about 10 seconds after fibrin injection before rising to the maximum level, while one animal showed no rise but a steady decline in pressure throughout the experiment. Meanwhile the right auricular pressure, which normally lies between −70 and −130 mm. saline, increased gradually to between +40 and +80 mm. saline and persisted at this level till death. The charts display clearly the variations in respiratory movements described above.

Our control experiments included the intracisternal injection of human and rat plasma, serum, heparinised blood, normal saline, thrombin solution (diluted 1 in 5) and fibrinogen (1 in 10), into both heparinised and non-heparinised animals, and it will suffice to state that in no instance did we encounter pulmonary œdema. A further valuable control experiment was performed by accident when we injected into two rabbits the fibrin-forming mixture to which had been added a rather large amount of prussian blue reagent. This mixture failed to coagulate after its introduction into the cranium and no

Fig. 2.—Kymographic tracing of respiration (R), carotid pressure (C.P.) and right auricular pressure (A.P.) in a rabbit before and after cisternal injection of 3 c.c. normal saline. Arrow indicates time of injection of saline.

pulmonary œdema was found after death. Nevertheless, respiratory responses and pressure changes in the carotid artery and right auricle developed in all controls as in rabbits with œdema of their lungs (fig. 2). The significance of this observation will be discussed later.

Pulmonary œdema also developed after the intracisternal injection of whole blood, human or animal, if coagulation was not prevented, and after injection of particulate suspensions such as India ink. Results were variable, but the mechanism seems to be very similar to that concerned with fibrin.

The outcome of these experiments is that we were able to produce a quickly developing, often fatal variety of pulmonary œdema by introducing into the cisternæ around the brain insoluble suspensions or particulates. It seems unlikely that any of the introduced material was absorbed into the blood stream during the short time which elapsed between its injection and the appearance of œdema, while there is evidence that in the case of fibrin-forming mixture none of the components (fibrinogen, thrombin, fibrin) injected into extracranial tissues such as the peritoneum, even in much larger amounts than we used for cisternal injection, induced œdema of the lungs. The stimulus for œdema production thus appears to be connected with the cranial contents, most probably the brain.

A neurogenic origin of the œdema

That we may ascribe this form of pulmonary œdema to a central nervous origin seems to follow from the results of experiments in which both vagi were divided shortly before injecting fibrin intracisternally. Eight out of nine rats so treated failed to show any œdema of their lungs when examined 15-30 minutes after injection, nor did they display clinical symptoms indicative of acute lung impairment. The ninth rat died immediately after cisternal injection and its lungs appeared to be normal. Ten rabbits were also investigated, using the same technique, pressure recordings being made on them. Six rabbits died after the usual interval. They displayed the same type of respiratory and blood-pressure reactions as animals with intact vagi, their carotid pressure rising and then falling, while their right auricular pressure rose steadily and persisted at a high level (fig. 3).

The effects of vagotomy can be seen in the charts before fibrin was injected intracisternally, being manifested as a slight transitory fall in arterial and auricular pressures, and by slowing and sometimes deepening of respiration. In none of these six rabbits was there any evidence of pulmonary œdema : the other four developed transient apnœa, but breathing was soon resumed, though of a slower and deeper character. Their carotid pressure fell below normal for a short period, then rose 30-50 mm. Hg. above the original level, remaining high in three cases for ten minutes, when they were killed. The fourth rabbit's carotid pressure returned to the normal range within six minutes of the initial injection. The right auricular pressure of all four animals rose as in non-vagotomised rabbits, though not to the same extent. None of the four showed œdema of the lungs.

Much the same results were obtained in rats pre-medicated with atropine sulphate (0·25 mg. per 200 g. rat intraperitoneally), which paralyses the vagal nerve endings. Nine out of the ten rats so treated 15 minutes before fibrin was injected into the cisternæ failed to develop pulmonary œdema ; one showed fairly severe œdema. Large doses of cocaine hydrochloride (40 mg. per 200 g. rat administered intraperitoneally) also gave astonishingly good results, 13 of 15 rats showing no œdema ; the same amount of cisternal fibrin produced œdema
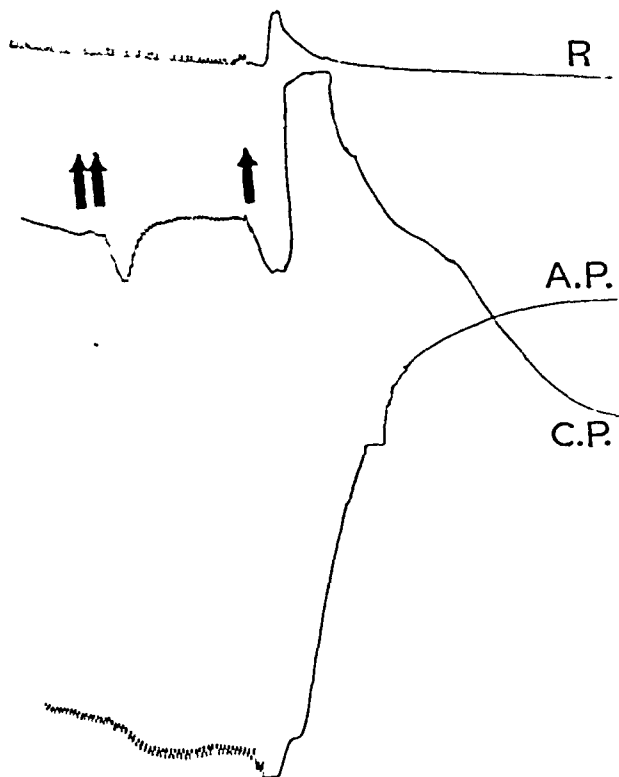
FIG. 3.—Kymographic tracing of respiration (R), carotid pressure (C.P.) and right auricular pressure (A.P.) in a rabbit before and after double vagotomy (two arrows) followed by cisternal injection (one arrow) of 3 c.c. fibrinogen-thrombin mixture.

with great regularity in untreated rats. It is possible that this effect of cocaine was due to depression of the nervous system, but we are still vague about the mode of action of the drug.

These experiments show that if the lungs are released from their vagal connections with the central nervous system their blood vessels are no longer vulnerable to certain stimuli which influence fluid exchange across their endothelial membranes.

We have tried to discover a structural explanation for this type of neurogenic œdema by studying the distribution of fibrin mixed with prussian blue after its introduction into the basal cisternæ. The

blue fibrin clot extends as a film on the sides of the brain stem, which thickens when it reaches the cisterna pontis and interpeduncularis (fig. 4). It stretches along the lateral recesses and forms a mass in the fourth ventricle below the tufts of the choroid plexus, conforming to the shape of the ventricular floor. Thick sections at appropriate levels show that the fibrin clot coats the surface of the vagal nuclei where these bodies project into the fourth ventricle (fig. 5), and it is possible that stimuli may be initiated by the fibrin-vagal nucleus
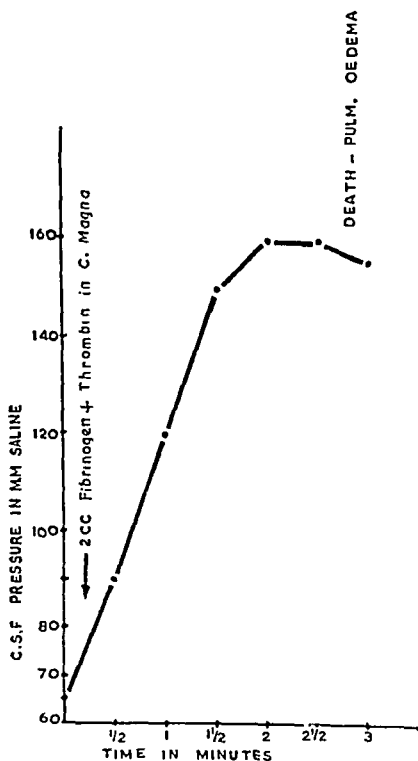
FIG. 6.—Rise of cerebrospinal fluid pressure in rabbit after cisternal injection of 3 c.c. fibrinogen-thrombin mixture.
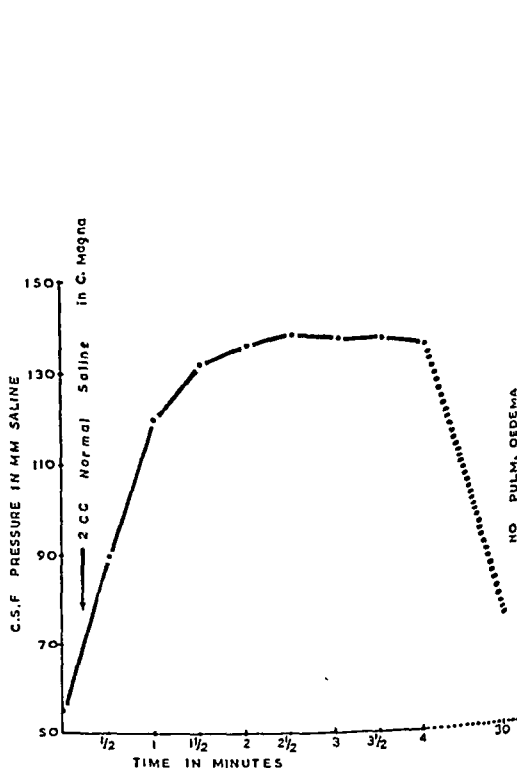
FIG. 7.—Rise of cerebrospinal fluid pressure in rabbit after cisternal injection of 3 c.c. normal saline.

contact. But the clot also extends into the aqueduct of Sylvius and along the third and lateral ventricles, while a very thin film may sometimes be traced across the sides of the cerebral hemispheres. Apparently there is no extension of fibrin along the perivascular spaces into the brain substance. Though it is tempting to refer the mechanism of œdema production to the vagal nucleus and to see in the contact between nucleus and fibrin a mechanical basis for the phenomenon, it must be admitted that the idea is no more than conjectural ; the widespread distribution of the clot allows of a number of alternative hypotheses which we have not been able to test. One possibility,

EXPERIMENTAL PULMONARY ŒDEMA

FIG. 4.

FIG. 5.

Fibrin in
fourth
ventricle
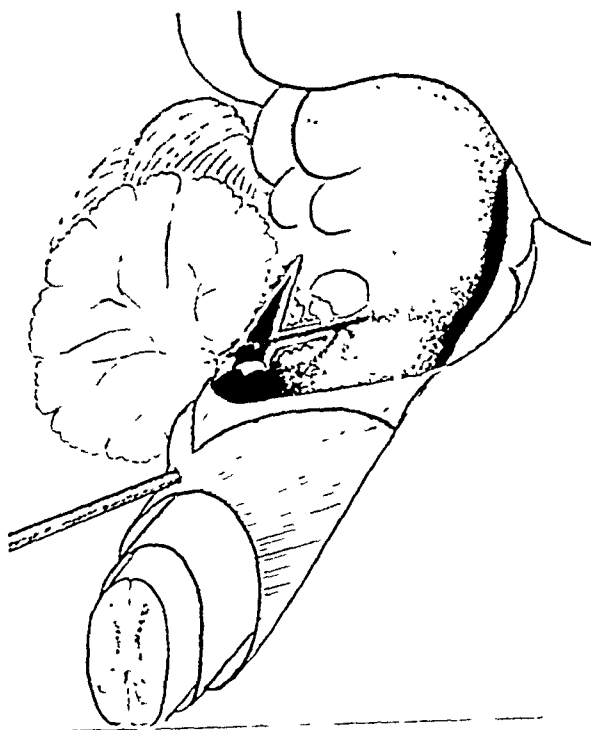
Fibrin in
cisterna
pontis
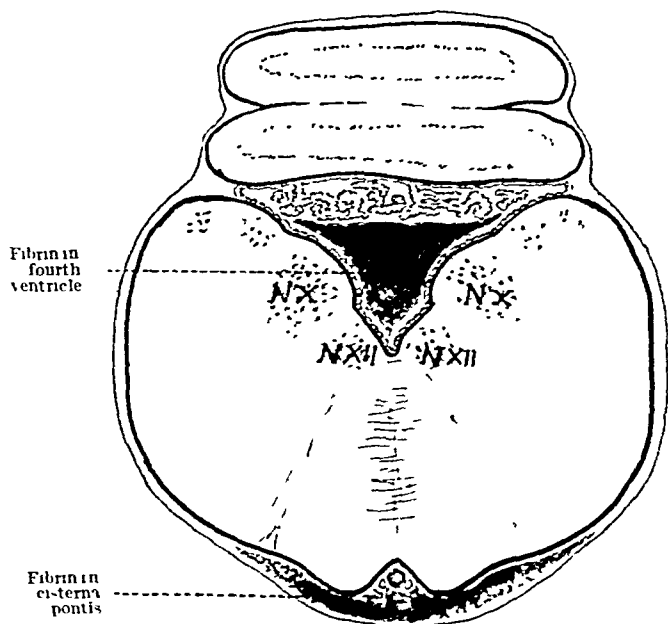
FIG. 4.—Diagram to illustrate the distribution of fibrin (black) in the basal cisternæ,
lateral recesses and fourth ventricle after cisternal injection. A portion of the
cerebellum has been removed to show the fourth ventricle. Injection needle at
left lower corner.

FIG. 5.—Diagram illustrating the relation of the fibrin clot to the dorsal nucleus of
the vagus nerve (NX) in the floor of the fourth ventricle. Frontal section.

however, can be excluded. There is a general impression amongst clinicians that a sudden rise of intracranial pressure may precipitate an attack of acute pulmonary œdema and indeed there is experimental evidence that this is correct (Benassi, 1937). We have recorded the cerebrospinal pressure of rabbits after cisternal injection of the fibrinogen-thrombin mixture and of corresponding amounts of normal saline. Pressures were noted at short intervals by means of a lumbar-puncture needle inserted into the subarachnoid space of the lower lumbar spine and connected to a saline manometer. Figs. 6 and 7 depict two typical experiments. Pressure variations were almost identical after both types of injection, yet one (the fibrinogen-thrombin mixture) led to pulmonary œdema and the other (saline) gave no such response. Obviously a rise of intracranial pressure cannot be blamed for the neurogenic œdema of the lungs which is a sequel to cisternal injection of fibrin.

The mechanism concerned in neurogenic fibrin œdema

Our efforts to find out the mechanism concerned in fibrin œdema include studies of the effects of severe tracheal obstruction, pulmonary embolism and certain drugs. Some investigators of pulmonary physiology (Short, 1944; Drinker, 1945) maintain that anoxia, especially when prolonged, is an important factor in the production of pulmonary œdema and this possibility must be considered as part of our problem, since vagal stimulation leads to bronchial constriction with diminution of the airway to the lungs and presumably some degree of anoxia. Evidence for an association between fibrin œdema and vagal function has already been given. However, we found no trace of pulmonary œdema in rabbits even after extreme tracheal obstruction for periods of time comparable with those concerned in fibrin œdema. Yet arterial and right auricular pressure variations were identical in the two cases.

We also wondered whether nervous factors might bring about lung œdema through interference with blood flow or pressure within the pulmonary vessels, despite the many uncertainties which still prevail about this subject (Daly, 1933; Cameron, 1948). But the most severe degrees of pulmonary embolism produced by injecting starch granules into the veins gave no evidence of œdema, although the catastrophic fall of carotid arterial pressure associated with a corresponding rise of right auricular pressure and an empty left heart at autopsy indicated almost complete interruption of pulmonary blood flow (fig. 8). Finally we tried to produce œdema by stimulating the peripheral ends of both vagi electrically, but in this endeavour we were unsuccessful.

DISCUSSION

The main contribution of our investigation is the demonstration that a rapidly progressive type of pulmonary œdema follows intra-

cisternal injection of a variety of unrelated substances, chief amongst which is fibrin. We have shown that the stimulus for œdema production arises within the central nervous system, for the initiating substances do not produce their effect when introduced extra-cranially, for instance, into the peritoneum, and they cease to be active when both vagus nerves are divided. It would appear that stimuli streaming from the central nervous system exert an action on the capillaries of the lungs whereby the normal equilibrium between hydrostatic pressure, osmotic pressure of blood plasma and tissue pressure is disturbed and plasma or even whole blood is poured out in large
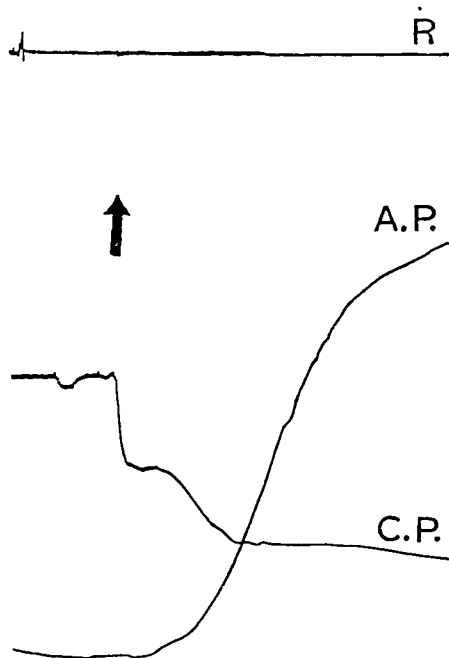
FIG. 8.—Kymographic tracings of respiration (R), carotid pressure (C.P.) and right auricular pressure (A.P.) before and after the intravenous injection of starch suspension. Arrow indicates time of starch injection.

amounts into the air spaces, terminating life by acute asphyxia. The exact mode of action on the cerebral tissues has not been discovered, though we favour medullary excitation, in particular of the region of the dorsal nucleus of the vagus nerve in the floor of the fourth ventricle as the immediate locus of activity. But the distribution of the irritating material and its film-like nature in the case of fibrin suggest that the effect may be a much more complex one and that a widely dispersed action in the neighbourhood of the ventricular system of the brain cannot be excluded.

Even more difficult is the analysis of the peripheral action of stimuli streaming from the brain to evoke the circulatory disturbance within the lungs. Can this be a direct action on the lung vessels, a truly

neurogenic effect mediated by the liberation of hormones from nerve endings, or is the process secondary to disturbances of other functions of the lungs, especially oxygen exchange ? More important still. can the pulmonary œdema be referred to circulatory failure because of vagal action on that system ? Unfortunately this aspect of the problem still suffers from many obscurities (see Cameron, 1948, for discussion) ; indeed, it is almost impossible to separate the circulatory and pulmonary complexities of lung œdema, but we believe that a case can be made out against a primary anoxic effect and a circulatory disturbance.

1. We have shown that severe respiratory obstruction, pushed to the extremes of asphyxia for a period of time equivalent to or even greater than that in action during the experimental neurogenic œdema resulting from intracisternal injection of fibrin, does not give lung œdema. This agrees with the observation that animals and man subjected to very low oxygen tensions do not develop œdema of the lungs (Van Liere, 1942 ; Gibbs *et al.*, 1943).

2. Neither respiratory records nor recordings of arterial and auricular pressures differ in animals which develop œdema after fibrin has been injected intracisternally or in those which show no œdema after the same procedure.

3. Vagotomy prevents œdema from developing, but does not interfere with the rise and fall of carotid pressure and the steady rise of right auricular pressure. In other words, œdema appears to be independent of what is going on in the pulmonary and systemic circulations.

4. Œdema commences very soon after the initiating stimulus has been set going, at a time when both arterial and auricular pressures are increasing and pulmonary circulation is being maintained. This suggests that it originates independently of any serious interference with the flow of blood through the lung capillaries.

For these reasons we are forced to consider the possibility of nerve stimuli which act directly upon the lung capillaries, making them more permeable to the plasma proteins. Evidence for a nervous factor which may influence permeability of blood vessels in general is scanty and too often contradictory ; we know of no satisfactory proof of such a principle in the case of the lungs. We have considered the possibility of acetylcholine being responsible for such an alteration in permeability because of its established position in the humoral transmission of parasympathetic nerve impulses, but apparently there is no reason for associating this compound with permeability increase. The recent investigations into the mode of action of drugs such as the fluorophosphonates which inhibit or destroy cholinesterase and lead to prolonged overactivity of acetylcholine within the animal (Adrian, Feldberg and Kilby, 1946 ; Dixon and Needham, 1946 ; Gaddum and Wilson, 1947) have not included pulmonary œdema among such toxic effects. One of us had the opportunity of studying

the pathological changes associated with fluorophosphonates during the late war and can vouch for the absence of acute œdema of the lungs. The pharmacological experiments of Cœlho and Rocheta (1933) also gave results in agreement with the above, since these workers introduced huge amounts of acetylcholine into the pulmonary artery of dogs without traces of œdema appearing. Dogs, too, tolerate large doses of histamine administered intrapulmonally (Cœlho and Rocheta). Indeed, the action of this compound on the lungs is comparatively slight and there is no reason to associate it with permeability changes therein (Drinker, 1948). These observations may be of some importance in narrowing the field of possible causes of increased capillary permeability.

Clearly further investigations are needed ; for the time being we must be content with a neurogenic hypothesis for the type of œdema we have been studying.

SUMMARY

Acute pulmonary œdema can be produced experimentally by cisternal injection of a fibrin-forming mixture. Whole blood or particulate suspensions such as India ink give similar though less constant effects. Records of the respiratory excursion, carotid blood pressure and right auricular pressure show that these undergo similar changes after severe tracheal obstruction, pulmonary embolism with starch granules and cisternal injection of fibrin with or without bilateral vagotomy, but only the last of these procedures is followed by acute pulmonary œdema. These results suggest that neither asphyxia nor mechanical disturbance in the pulmonary circulation plays any part in the genesis of the œdema. The stimulus for œdema production arises within the central nervous system, for the irritants cease to be active when they are injected extracisternally, when both vagus nerves are divided or their endings paralysed with atropine, or when cocaine is administered before the cisternal injection. The increased cerebrospinal fluid pressure which follows the cisternal injection is not the œdema-producing stimulus. It is suggested that the permeability of the lung capillaries may be modified by the outflow of stimuli from the brain stem by way of the vagus nerves whereby plasma leaves the pulmonary vessels on so large a scale as to induce œdema of the lungs.

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THE IDENTIFICATION OF *CLOSTRIDIUM ŒDEM-ATIENS* AND AN EXPERIMENTAL INVESTIGATION OF ITS ROLE IN THE PATHOGENESIS OF INFECTIOUS NECROTIC HEPATITIS ("BLACK DISEASE") OF SHEEP

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(PLATES LXXXVII-LXXXIX)

TECHNICAL obstacles to the study of anaerobes, though not inherently serious, are troublesome enough to have curtailed or delayed the investigation of many important anaerobic infections. This is particularly true of *Cl. œdematiens* infection, because the organism is not only more exacting in its growth requirements than *Clostridium welchii*, but is also much more difficult to identify with precision. For this reason among others, infectious necrotic hepatitis ("black disease") of sheep, although described in Australia by Gilruth as early as 1910, was not thought to be associated with *Cl. œdematiens* until Albiston (1927) suggested this organism as the bacterial cause. It had previously been known, however, that infestation with the liver fluke, *Fasciola hepatica*, was in some way necessary for the production of the disease (Dodd, 1918a and b, 1921). In Great Britain, *œdematiens* infections in animals were rarely reported (Bosworth and Jordan, 1929 ; Roberts and McEwen, 1931 ; McEwen, 1931), although a condition known for many years in the north of Scotland as " watery braxy " of sheep was shown to be black disease (Jamieson *et al.*, 1948).

The purpose of this paper is to record studies of the strains of *Cl. œdematiens* isolated from the lesions of black disease, the latent distribution of the organism in different hosts and the experimental reproduction of the disease.

TECHNICAL METHODS

Strains. Seventeen strains of *Cl. œdematiens* isolated from the hepatic lesions of black disease were examined in detail. Twenty-eight strains obtained from the liver of apparently healthy sheep, cattle, rabbits and one crow were less fully investigated. Three type-A strains from human and bovine hosts, C.N. 637, C.N. 1780 and C.N. 929 from the Wellcome Laboratories' collection, were

compared with the newly isolated strains. The most effective method of isolation was to inoculate Robertson's cooked-meat medium with material taken from the edge of a necrotic hepatic lesion, *i.e.* from the region of the leucocyte barrier between the necrotic lesion and normal liver tissue. Forty-eight-hour cultures in this medium were then inoculated into a series of glass tubes about 15 cm. long and 5 mm. in diameter containing Weinberg's " viandl-foie " (V.F.) agar, as described by Turner (1930).* Each series covered a range of tenfold dilutions to 10^{-9} and pure cultures were usually obtained from the highest dilution showing growth.

Identification by toxin-antitoxin tests on solid media. The irregularity of growth on solid media of *Cl. œdematiens* recently isolated from animal hosts is well known, but toxin-antitoxin tests on solid media can be used at an early stage of identification. It was found that a method based on the recommendations of Petrie and Steabben (1943) was highly successful. The method was modified so that the colonies grew immediately below the surface, and the antitoxin concentration was reduced from 75 to 4 I.U. per ml. of medium. In a positive test (fig. 1) the colony is surrounded by irregular zones of varying opacity.

Media. Various media were examined for their ability to support good continuous growth of subcultures. Proteose-peptone 2·5 per cent. with 0·5 per cent. glucose in distilled water proved the most consistent and reliable. The medium was used in 100-ml. quantities, freshly autoclaved. Optimum growth was observed between pH 8 and 7·8 ; outside this range results were poor. During growth of the organisms the pH of the medium fell at a rate roughly proportional to the speed of growth, *e.g.* from 8·0 to 5·4 in liver-saline medium within 4 days.

Fermentation reactions. The method which proved most useful was that of Reed and Orr (1941). Tubes with 5 ml. of their semi-solid proteose-peptone medium containing 1 per cent. of fermentable substrate were inoculated from a vigorous 48-hour proteose-peptone culture and incubated at 37° C. for two days in a McIntosh and Fildes jar. All strains produced acid in glucose and some produced acid in maltose. Lactose, sucrose, mannitol, salicin and glycerol were not changed by any of the strains examined.

Spore suspensions. Bacterial suspensions in which 80 per cent. of the organisms carried spores were obtained by alternate pasteurisation and sub-culture. The procedure was to heat cultures in Robertson's cooked-meat medium at 100° C. for 3 minutes and transfer 0·05 ml. to a fresh tube containing 10 ml. of the same medium which was then incubated at 37° C. for 4 days in a McIntosh and Fildes jar. From this, spore suspensions were prepared in bulk by using the 10 ml. of 4-day culture to inoculate 500 ml. of the same medium. This was incubated for 4 days at 37° C. in the anaerobic jar and allowed to stand for 14 days on the bench at room temperature. The spores were washed free from toxin by centrifuging thrice with glass-distilled water, and the deposit was suspended in saline to give an opacity four times that of Brown's tube no. 10 and heated for 30 minutes at 80° C. to kill vegetative forms. The total spores were counted by Breed's slide method and the number of viable spores was estimated roughly by inoculating 0·05 ml. of serial dilutions down to 2^{-10} into 10 ml. of cooked-meat medium. The suspensions were stored in the cold at $\pm 5°$ C. and remained viable for at least 6 months.

Lecithinase differentiation

Preliminary identification. The differentiation of *Cl. œdematiens* from other anaerobes and types A and B from each other by their production of specific lecithinase was described by Oakley *et al.* (1947) and their methods were largely

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* See appendix for details of preparation of the medium.

used in the present work. When the cultures under investigation had been brought to a reasonably pure state a preliminary identification test based on lecithinase production proved successful. The test consisted of the titration of supernatant fluids or filtrates of the culture against a single dilution of *œdematiens* anti-β and anti-γ sera and of *welchii* anti-α serum. One ml. of the filtrate was mixed with 1·0 ml. of saline, and 0·5 ml. of lecitho-vitellin (L.V.) suspension was added. A control tube was set up with 2·0 ml. of saline and 0·5 ml. of L.V. suspension. Both tubes were placed in a water-bath at 37° C. for one hour. The presence of a lecithinase was indicated by the development of opalescence in the tube containing filtrate but not in the saline control.

To identify the lecithinase present, 1·0 ml. of the filtrate was then added to three tubes containing 1·0 ml. of antiserum diluted to contain 20 units respectively of *œdematiens* β and γ antitoxin and of *welchii* α antitoxin, and after half-an-hour at room temperature 0·5 ml. of L.V. suspension was added and the tubes were incubated at 37° C. in the water-bath for 1 hour. The possible results and their interpretations are given in table I.

TABLE I

Possible results from method of preliminary testing for specific lecithinases

Result no	Tube I (*œdematiens* anti-γ serum)	Tube 2 (*œdematiens* anti β serum)	Tube 3 (*welchii* anti α serum)	Probable lecithinase in filtrate
1	—	±	—	Œdematiens γ (type A)
2	±	—	—	,, β (type B)
3	±	±	—	Welchii α
4	±	—	±	Possibility of Cl. hæmolyticum or other bacterial species (Oakley et al., 1947; McGaughey and Chu, 1948)

± = opacity in L.V. suspension
— = no opacity in L.V. suspension

For the present work further tests were devised for confirmation of type lecithinase and to estimate the variation of lecithinase production in different media.

Type lecithinase confirmation tests. The range of antiserum dilutions in confirmation tests to differentiate *œdematiens* types A and B depends on the dilution used in the preliminary lecithinase identification test, which in the present examinations was 7·5 units of β antitoxin.

Test 1. Titrations were made with 1·0 ml. of filtrate against 7·6, 5·0, 2·0, 1·0 and 0·1 β units of antitoxin. After these had stood on the bench for half-an-hour, 0·5 ml. of L.V. suspension was added as indicator to each tube and the tubes were placed in a water-bath at 37° C. for exactly one hour, after which they were examined for opacity in the usual way.

Test 2. In test 1, the first dilution of antiserum showing opalescence was usually in the range 1·0–0·1 β unit. The actual figure was used as a guide in preparing a further range of dilutions at closer intervals. This procedure was repeated twice with two sera : 0·5 ml. of L.V. suspension was added to each serum dilution and the rest of the procedure carried out as in test 1.

Test 3. From the results of test 2 a close range of dilutions of filtrate was made and titrated against a constant antitoxin dilution of 0·1 β unit. The filtrate dilutions ranged from 1·5 to 0·10 ml., an example of the usual spacing being 0·4, 0·36, 0·2, 0·15, and 0·10. These tests were replicated with two β antisera, R.X. 5325 and E.X. 117.

L.V. suspension prepared on the w/v basis recommended by McGaughey and Chu (1948) proved most reliable. Filtrates for these examinations were obtained by inoculating 100-ml. amounts of 2·5 per cent. freshly autoclaved proteose-peptone containing 0·5 per cent. glucose with vigorous actively growing cultures in cooked-meat medium. The proteose-peptone cultures were then incubated for 4 days at 37° C. in the anaerobic jar. The cultures were centrifuged at 7000 r.p.m. for 15 minutes and the supernatant used as the " filtrate."

Lecithinase production tests

These tests were used to determine the growth conditions for maximum lecithinase production. The tests involved preparation of two series of two-fold dilutions down to 2^{-10} in Lambeth tubes measuring 8×0.75 cm.

Series 1. An initial dilution of antitoxin was made to yield 20 β units per ml. One ml. of this dilution was added to tube 1 of a series of ten tubes containing 1 ml. of 0·85 per cent. saline. From tube 1 two-fold dilutions were extended along the series. To each tube 1 ml. of filtrate was added.

Series 2. This consisted of a series of ten tubes each containing 1 ml. of 0·85 per cent. saline. To tube 1, 1 ml. of filtrate was added and two-fold dilutions were continued to the end of the series.

0·5 ml. of L.V. suspension was added as indicator to each tube in both series and the tubes placed in the water-bath at 37° C. for one hour. Readings were made at this interval and again 12 hours later.

Cultivation of cercariæ

Cercariæ of *Fasciola hepatica* were cultivated in the laboratory according to the technique of Taylor and Mozley (1948) and were administered by mouth to guinea-pigs and rabbits in gelatine capsules containing wet bran.

EXPERIMENTAL OBSERVATIONS

Identification of Cl. œdematiens

In establishing the post-mortem diagnosis of black disease of sheep it was necessary to prove the identity and type of strain of *Cl. œdematiens* cultured from diseased livers. Cultures were made as already described ; thereafter procedure and results were as follows.

Preliminary identification. The toxin-antitoxin reactions of *Cl. œdematiens* on the surface of solid medium have been recommended as a rapid and reliable means of identifying type-A strains of *Cl. œdematiens* (Petrie and Steabben, 1943 ; Nagler, 1945 ; Oakley *et al.*, 1947). Petrie and Steabben tested only one type-A strain, however, and it seemed necessary to check their observation by examining not only further strains of type A but also strains of type B. With all my strains, 3 of type A and 17 of type B, the modified method proved a useful means of differentiating *Cl. œdematiens* from other anaerobes, but it did not of course distinguish type A from type B. With strains of type B isolated from animal hosts, preliminary identification on the surface of solid media as suggested by Oakley *et al.* did not prove so reliable because of the uncertain growth obtained from recently isolated type-B strains.

When the preliminary identification was made by examining for lecithinase activity by the methods recommended by Oakley *et al.*, all my strains appeared to be type B, with inhibition of opalescence by 1·0 ml. of filtrate in the presence of 7·5 units of β antitoxin. Further evidence had to be obtained, however, since Oakley *et al.* demonstrated that qualitative examination of a filtrate could not be regarded as proof that this contained β or γ toxin alone ; they maintained that it was necessary to show in type-confirmation tests that toxin in the filtrate was neutralised by several sera in proportion to their antitoxin content. They quoted the failure of β or γ antitoxin to inhibit opalescence in filtrates from two strains of *Cl. hæmolyticum* before dilution as demonstrating the necessity for these tests.

Type-confirmation tests. Such type-confirmation tests were accordingly applied to my strains with the results shown in table II. These

TABLE II

Results of confirmatory type-lecithinase tests for Cl. œdematiens

Strain			Volume of filtrate (ml.) neutralised by 0·1 unit β antitoxin	Strain	Volume of filtrate (ml.) neutralised by 0·1 unit β antitoxin
Glengolly	.	.	0·4	B.D.J./19	0·4
B.D.J./1	.	.	0·15	B.D.J./20	0·10
B.D.J./2	.	.	0·4	B.D.J./21	0·15
B.D.J./6	.	.	0·4	B.D.J./25	0·2
B.D.J./9	.	.	0·2	B.D.J./26	0·15
B.D.J./11	.	.	0·7	B.D.J./27	0·4
B.D.J./12	.	.	0·8	B.D.J./28	0·10
B.D.J./14	.	.	0·4	B.D.J./35	0·4
B.D.J./18	.	.	0·2	...	...

results show the volume of toxin-containing filtrate completely neutralised by 0·1 unit of β antitoxin and they afford proof that the filtrates are neutralised by antitoxic sera in proportion to their β antitoxin content and therefore contain β toxin. This establishes that the strains are *Cl. œdematiens* type B.

OPTIMUM GROWTH CONDITIONS FOR PRODUCTION
OF LECITHINASE

There are inherent difficulties in the expression of an end result when testing for variations in the lecithinase activity of filtrates. The two main difficulties are the variability of the crude L.V. suspension and the reduction of the final dilutions to absolute points. To overcome these difficulties methods were devised which had the advantage that the test filtrate was examined simultaneously in the presence and in the absence of antitoxin. As the strains were shown by the type-confirmation tests to belong to type B, it was sufficient to include a single tube with 150 units of *œdematiens* γ antitoxin and 1 ml. of

TABLE III

Influence of various media on β toxin (lecithinase) production by four strains of Cl. oedematiens, type B

Result of adding culture filtrate to L V suspension plus. Antitoxin columns give *Oedematiens* β antitoxin (units), *Oedematiens* γ antitoxin (150 units) [*], and Saline (ml filtrate).

Medium	Strain	Final pH	β 20	β 10	β 5	β 2 5	β 1 25	β 0 6	β 0 3	β 0 15	β 0 08	β 0 04	β 0 02	γ 150	sal 0 5	sal 0 25	sal 0 13	sal 0 06	sal 0 03	sal 0 02	sal 0 01	sal 0 005	sal 0 003	sal 0 001
2 5 per cent proteose-peptone +0 5 per cent glucose	11	7 0	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	12	5 8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	−	−
	21	6 0	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	−	−	−	−
	35	5 8	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
4 per cent peptone +0 5 per cent glucose	11	6 2	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	12	6 4	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	±	−	−	−	−	−	−
	21	6 5	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	35	6 3	−	−	−	−	±	+	+	+	+	+	+	+	+	+	±	−	−	−	−	−	−	−
4 per cent peptone +1 per cent glucose	11	6 2	−	−	−	±	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
	12	6 4	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	21	6 6	−	−	−	±	+	+	+	+	+	+	+	+	+	+	±	−	−	−	−	−	−	−
	35	6 0	−	−	−	−	±	+	+	+	+	+	+	+	+	+	±	−	−	−	−	−	−	−
4 per cent peptone +2 per cent glucose	11	6 4	−	−	−	−	−	−	−	−	±	+	+	+	±	−	−	−	−	−	−	−	−	−
	12	5 6	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	21	6 5	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	35	6 3	−	−	−	+	+	+	+	+	+	+	+	+	+	+	±	−	−	−	−	−	−	−
Brewer's	11	6 3	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
	12	6 2	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
	21	6 2	−	−	−	−	−	−	−	+	+	+	+	+	+	±	−	−	−	−	−	−	−	−
	35	6 1	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−
4 per cent peptone +10 per cent toxin factor (horse muscle)	11	6 8	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
	12	6 8	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
	21	6 8	−	−	−	−	±	+	+	+	+	+	+	+	+	+	±	−	−	−	−	−	−	−
	35	6 8	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Liver saline	11	5 5	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−
	12	5 4	−	−	−	−	−	−	−	−	−	−	−	+	+	±	−	−	−	−	−	−	−	−
	21	5 4	−	−	−	−	−	−	−	−	−	−	−	+	+	+	±	−	−	−	−	−	−	−
	35	5 4	−	−	−	−	−	−	−	−	−	−	−	+	+	±	±	−	−	−	−	−	−	−

+ = opacity in L V suspension   ± = trace of opacity in L V suspension   − = no opacity in L V suspension

* The failure of γ antitoxin to neutralise the reactions shows that the *oedematiens* strains are not of type A

filtrate. Since this large amount of γ antitoxin did not inhibit their lecithinase activity the strains could not belong to *Cl. œdematiens*, type A.

Variations in lecithinase production in different media proved troublesome in the earlier examinations. Because of this, four strains were examined in 7 different media with the results detailed in table III, from which it is obvious that the differences observed were due to growth conditions and not to strains. It appears significant that where growth was rapid, as in liver-saline, lecithinase production was barely detectable.

NATURAL INCIDENCE OF LATENT SPORES

From 1945 to 1948 a survey was made in the North of Scotland to determine the presence or absence of latent spores of *Cl. œdematiens* in the liver of apparently healthy sheep grazing pastures on farms where black disease had been diagnosed, and on farms where black disease had not been reported.

Since crows and lesser gulls are attracted to sheep dead from black disease the survey was extended to include the liver from these birds. The isolation of *Cl. œdematiens* from the beaks of crows by Edgar (1931) and from a swan dead of a septicæmic disease (Piening, 1932) supported the belief that latent *Cl. œdematiens* spores might be found in the tissues of birds.

Although the number of examinations may not be large enough to justify more than tentative conclusions, two significant points emerge from the results (table IV) : first, that sheep grazing in black-disease

TABLE IV

The incidence of latent spores of Cl. œdematiens *in the liver of apparently healthy animals in black-disease areas and in areas from which the disease is absent*

Host species	Black-disease areas			Other areas		
	No. of liver-examined	Livers with spores		No. of liver-examined	Livers with spores	
		No	Per cent		No	Per cent
Sheep	38	11	28·9	26	0	0
Cattle	8	6	75 0	15	1	6·7
Starlings	24	0	0	12	0	0
Gulls	8	0	0	2	0	0
Crows	10	1	10 0	10	0	0
Rabbits	35	3	8 6	11	0	0
Totals	123	21	17·1	76	1	1 31

areas have a much higher incidence of latent *œdematiens* spores in the liver than those grazing elsewhere ; and second, that there is a relatively high incidence of latent spores in the liver of cattle, suggesting that black disease probably affects bovines in this country.

Experiments to show spore latency

Expt. 1. Six guinea-pigs, three rabbits and one sheep were given doses of washed spores of *Cl. œdematiens* by the routes shown in table V. Guinea-pig no. 3 became ill two days after receiving the spore suspension by mouth and died on the fourth day. Post-mortem examination revealed the typical appearances which follow the ingestion of whole cultures of *Cl. œdematiens* : a congested, hæmorrhagic gastro-intestinal tract and gelatinous effusions into the serous cavities. The dose of

TABLE V

The distribution of latent Cl. œdematiens *spores in animal tissues after their experimental introduction (experiment 1)*

Animal	Route by which spores were given	Dose of spores (millions)	Day of experiment on which killed	Presence (+) or absence (−) of *Cl. œdematiens* in			
				liver	spleen	bone marrow	muscle
Guinea-pig no. 1	Intravenous	25	50	+	+	−	−
,, ,, 2	,,	10	50	+	+	−	−
,, ,, 3	Oral	200	4 (died)	+	+	+	+
,, ,, 4	,,	100	50	+	−	−	−
,, ,, 5	Intraperitoneal	10	50	+	+	−	−
,, ,, 6	,,	5	50	−	+	+	−
Rabbit no. 1 .	Intravenous	25	50	−	+	−	−
,, ,, 2 .	Intraperitoneal	15	50	+	−	−	−
,, ,, 3 .	Oral	100	50	+	+	+	−
Sheep . .	Intramuscular	10	60	+	+	−	+

spores was certainly high, but there was no evidence that vegetative forms were present, and the same suspension was used intravenously in guinea-pig no. 2 without ill effect. All other animals remained well until killed. Spores were more often found in the spleen and liver than in the bone marrow or muscle.

Expt. 2. Two rabbits were each given two intravenous injections of 2,500,000 spores in saline with an interval of 8 days between the injections. Seven days after the last injection 10 cercariæ of *Fasciola hepatica* were given by the mouth to one rabbit and 15 cercariæ to the other. Twenty-one days later both rabbits appeared well and they were then killed with chloroform and examined. In both there was evidence of liver-fluke infestation but there were no necrotic lesions resembling those seen in black disease of sheep. Vegetative bacillary forms of *Cl. œdematiens* were not found in smears or sections of the liver. From the liver and spleen of both rabbits *Cl. œdematiens* was isolated in pure culture.

Expt. 3. One rabbit was given 5 million spores by one intravenous injection and another received 40 million spores in a single dose by mouth. These two rabbits and a control which had received no spores were given each 25 cercariæ. Ten days later the rabbit died which had received the spores intravenously. Post-mortem examination

showed that the liver was acutely congested and was the site of a single hæmorrhagic rupture of the capsule. No necrotic lesions were observed on the surface or in the substance of the organ. From the liver two immature liver flukes were recovered. *Cl. œdematiens* was isolated in pure culture from the liver and spleen.

At the end of 36 days the other two rabbits of the experiment were still healthy. Five million spores were now given to each rabbit intravenously in a single injection. Ten days later the animals, which were still healthy, were killed by chloroform. Liver-fluke infestation was present in each but there was no evidence of necrotic lesions resembling black disease. *Cl. œdematiens* was isolated from the liver in both animals.

Expt. 4. A guinea-pig weighing 900 g. received toxin-free spores of *Cl. œdematiens* to a total of 5 million by the intraperitoneal route. Spores were given on 19th, 22nd, and 29th January 1949, in doses of 1, 1½ and 2½ millions. Twenty days later (18th February), 25 cercariæ were given by mouth. Eighteen days later the animal died after an illness of less than 2½ hours. Post-mortem examination revealed the general features typically observed in sheep dead of black disease, namely distension of the pericardium with clear fluid, zonal congestion of the pylorus and the presence of clear fluid in the peritoneal cavity. The liver showed numerous necrotic tracks and two small necrotic areas about 3 mm. in length situated in a zone of congestion. The necrotic areas were sharply demarcated by an intense bright red zone. Histological examination of the necrotic areas revealed necrosed hepatic cells surrounded by a leucocytic barrier (fig. 2) in which vegetative bacilli were evident (fig. 3). No bacilli were observed in the necrotic tracks made by the passage of flukes. *Cl. œdematiens* was isolated from the leucocytic barrier of these lesions.

Expt. 5. Expt. 4 was repeated with a guinea-pig which received in one dose 5 million *Cl. œdematiens* spores by the intravenous route and, 30 days later, 25 cercariæ by mouth. At the same time a control guinea-pig, not given spores, received 25 cercariæ by mouth. The guinea-pig which received both spores and cercariæ died 21 days after dosage with cercariæ, with the typical lesions of black disease as described in expt. 4. Seven days later the control guinea-pig, which had not received spores, was killed by chloroform; it showed evidence of fascioliasis only.

DISCUSSION

In the absence of a standard procedure and an accepted classification of *Cl. œdematiens* by fermentation reactions, the value of these reactions is severely restricted. Turner (1930), using Weinberg's V.F. broth rendered sugar-free by the growth of *Cl. welchii* and containing 10 per cent. of added fermentable substrate, concluded that strains isolated from cases of black disease were distinguishable

from strains of human and equine origin by the failure of the black-disease strains to ferment glycerol. Scott *et al.* (1934) recommended this difference as a basis for identification. Keppie (1944), on the other hand, using a medium containing 1 per cent. of horse serum and 0·5 per cent. of fermentable substrate, found three type-B strains which fermented glycerol. It is significant, however, that one of these strains was isolated from a horse and one from an ox, and that the origin of the third strain was unknown, whereas classical type-B strains " Gigas I," " Tongala " and " Albiston," all isolated from sheep, failed to ferment glycerol. Oakley *et al.*, using a medium essentially similar to that of Keppie, found that 10 of 14 type-B strains fermented glycerol. No information is given about the host origin of these strains. The fermentation reactions of my strains conform more closely to those of Turner than to those reported by Keppie and by Oakley *et al.* The point of interest is that all Turner's strains and all my 17 strains from sheep with black disease failed to ferment glycerol.

The rapid identification of *Cl. œdematiens* is more difficult with type-B than with type-A strains because of the fastidious growth requirements of type-B strains. The modified method of Petrie and Steabben proved the most reliable, and, supplemented by preliminary lecithinase tests, should prove invaluable in the diagnosis of anaerobic diseases of sheep and cattle, where mixed infections are common. The typing of strains by the use of specific anti-lecithinase sera proved satisfactory with my black-disease strains. The variation in lecithinase production in different media needs further investigation. Keppie, using the turbidimetric method of van Heyningen (1941), showed that with *Cl. œdematiens* lecithinase production was not related to production of the main lethal toxin as it is with *Cl. welchii*. He showed that the addition of extract of horse muscle and glucose were both important for the demonstration of even slight lecithinase production by type-B *œdematiens* strains. My observations (table III) show that the rate of growth of the strains, as judged by the lowering of pH in the culture, is not proportional to the production of lecithinase. Thus liver-saline medium, which invariably produced a low final pH and a rapid and luxuriant growth, was notable for low lecithinase production. Strains 12 and 35 generally yielded higher lecithinase levels than the other strains. The failure of lecithinase production in Brewer's semi-solid medium with 0·5 per cent. agar was not surprising. In preliminary studies with this medium, I often observed spurious reactions in duplicate parallel tests, replicated with different batches of L.V. suspension. Oakley *et al.* recommended Brewer's semi-solid medium for routine lecithinase testing, using high-speed centrifugation or filtration over Hyflosupercel (Johns-Mancille Ltd.) to obtain the filtrate. In my tests high-speed centrifugation gave obviously discrepant results, but these were corrected by growing the organism in Brewer's broth without agar, or by filtration through kieselguhr.

CL. ŒDEMATIENS IN BLACK DISEASE OF SHEEP

FIG. 1.—Positive toxin—antitoxin reaction with *Cl. œdematiens* type B on solid medium. Note irregular zones of varying opacity around the central pin-head colony (natural size).

FIG. 2.—Necrotic lesion surrounded by a barrier of leucocytes in the liver of a guinea-pig produced by experimental i-p. inoculation of *œdematiens* spores followed by cercariæ of *Fasciola hepatica* by mouth. Hæmatoxylin and eosin. ×20.
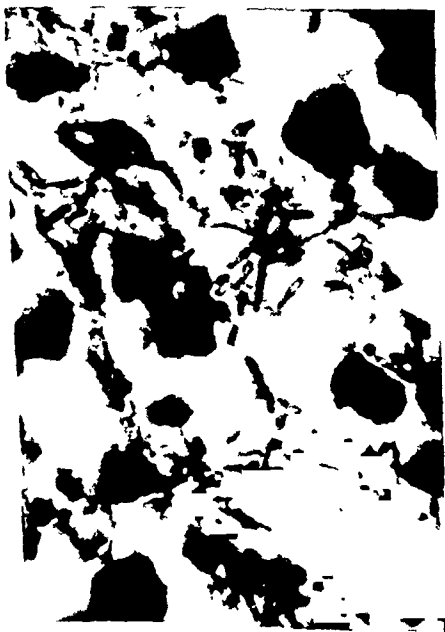
FIG. 3.—Higher-power view of fig. 2 to show vegetative and sporulating forms of *Cl. œdematiens* in the leucocyte barrier. Gram's stain. ×1200.

FIG. 4.—Lesion from natural black disease of sheep. Fluke track in liver ending at the edge of an infective necrotic lesion. H. and E. ×60.

CI. Œ DEMATIENS IN BLACK DISEASE OF SHEEP
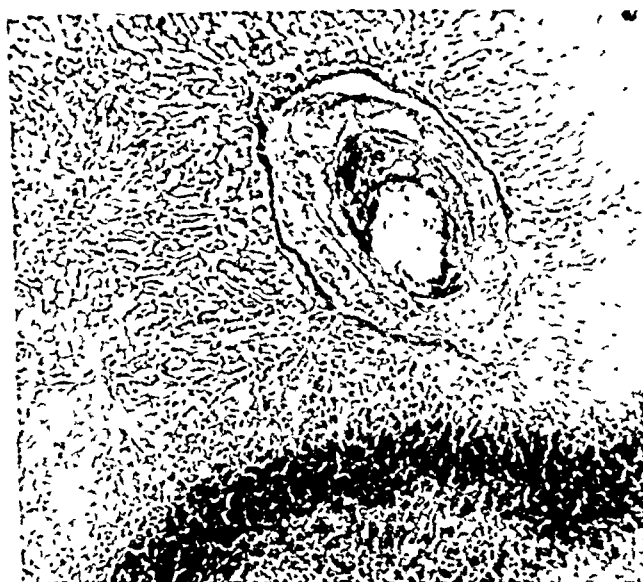
FIG 5 —Lesion from natural black disease of sheep   Remnants of fluke track within an area of hepatic necrosis which is surrounded by a dense zone of leucocytes (below)   ×60

FIG 6 —Vegetative *Cl œdematiens* from the leucocyte barrier of fig 5   Gram's stain   ×1200

FIG 7 —Lesion from natural black disease of sheep   Immature fluke in tissue adjacent to infective lesion   H. and E.   ×20.

The behaviour of strain 12 in 2·5 per cent. proteose-peptone plus 0·5 per cent. glucose medium suggests that other lecithinases might be present for which no anti-lecithinase existed in the sera ; alternatively the explanation might be that this particular lecithinase did not combine well with the antitoxin. The lecithinase tests which were devised, although not free from disadvantages, were more sensitive than turbidimetric estimations for *Cl. œdematiens* and greatly reduced the danger of introducing other factors which might give spurious results.

Since the work of Dodd (1918a and b, 1921), when black disease was first shown to have a dual parasitic and bacterial origin, the pathogenesis of this disease has attracted considerable interest. Turner (1928, 1929, 1930) believed that the disease was brought about by the activation of latent spores of *Cl. œdematiens* by the immature wandering liver fluke. The evidence in support of this hypothesis was indeed weighty. Histological studies of the liver in black disease unfailingly demonstrated the various stages of fluke infestation as well as the infective necrotic lesion, which consisted of necrotic liver cells surrounded by a deep leucocytic barrier in which vegetative bacillary forms were easily demonstrated (figs. 4-7). That black disease was found only in known areas of fluke infestation further supported Turner's view. The discovery of latent spores of *Cl. œdematiens* in the liver of apparently healthy sheep (Edgar, 1928 ; Turner, 1930) was additional evidence pointing to the probable method of infection.

In his experiments, Turner activated spores of *Cl. œdematiens* in the liver of guinea-pigs but his experimental method did not establish whether, in the natural infection, it was likely that the spores were taken in by the fluke or were already present. He fed 8,000,000 toxin-free spores to four guinea-pigs which, 85 days later, received 15 cercariæ of *Fasciola hepatica* by mouth. Twenty-two days later, the animals were still alive. A control guinea-pig given only cercariæ showed extensive liver damage by the young flukes. Turner concluded that the spore population in the four guinea-pigs was not adequate for production of the disease and accordingly·his four animals were each given 12,500,000 spores intraperitoneally. Within three days all four guinea-pigs died. A control guinea-pig which received 25,000,000 toxin-free spores showed only a passing indisposition, but recovered. On histological examination Turner recorded the marked necrosis along the fluke tracks and the presence therein of *Cl. œdematiens*. He stated that it did not necessarily follow that the spores actually grew along the fluke tracks.

Turner's experiments, however, do not prove that latent spores of *Cl. œdematiens* in the liver are activated by wandering liver flukes. All that is certain is that when toxin-free spores were introduced at the height of an acute fluke infestation of the liver, in an animal believed to be carrying latent spores, the guinea-pigs died, and that

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on post-mortem examination vegetative forms of *Cl. œdematiens* were found along the necrotic fluke tracks.

Certainly I have not seen a natural case of black disease in which vegetative bacilli were demonstrated in the necrotic tracks. It is significant that in the summary of a general paper on anaerobic infections in animals Scott *et al.* make the statement that black disease is due to the germination of latent spores of *Cl. œdematiens* which have been carried to the liver by wandering liver flukes. The question has hitherto been unanswered as to whether latent spores of *Cl. œdematiens* in the liver can be activated by young wandering liver flukes or whether the spores must be introduced after or along with the liver-fluke infestation. Expts. 4 and 5 of this paper show conclusively for the first time that the experimental disease in guinea-pigs can be produced by activation of latent spores by wandering flukes, and that there is no need to postulate that the *œdematiens* spores are carried into the liver by the flukes. This makes the pathogenesis of the natural disease much more intelligible.

In confirmation of the earlier Australian work (Edgar, 1928; Turner, 1930), the present paper shows that 28·9 per cent. of healthy sheep from black-disease areas have latent spores of *Cl. œdematiens* in the liver and that the organism was not isolated from the liver of any of the healthy sheep from areas free of black disease. The positive findings in the liver of rabbits, cattle and one crow in black-disease areas (table IV) provide supporting evidence of the association between black disease and the spores of *Cl. œdematiens* latent in the organs of animals.

Histological examination of the liver lesions from sheep with black disease confirms the Australian findings. The termination of a fluke track in a typical infective necrotic lesion (fig. 4) is evidence that wandering flukes activate already existing latent spores. The evidence indicates that when spores are activated a necrotic lesion is produced by the necrotising toxin liberated by *Cl. œdematiens*. The size of the lesion and the intensity of the leucocytic response obviously depend upon the number of spores activated, the potency of the toxin, whether the conditions are optimal for bacterial growth, and the defensive response of the animal. The diffusion of toxin must rapidly reduce the lining of the track and the surrounding tissue to a necrotic mass. The animal dies within a few hours and histologically it can be shown that the fluke track terminates in the necrotic lesion.

The disease was reproduced in two guinea-pigs, into which spores were experimentally introduced and to which cercariæ were fed after an interval of 21-30 days. The guinea-pigs died with the typical syndrome and the pathological changes associated with black disease in sheep. Histologically the lesions in the guinea-pig (figs. 2 and 3) were essentially similar to those of naturally occurring black disease (figs. 4-7). Minor differences in the guinea-pig lesions were the less

CL OEDEMATIENS IN BLACK DISEASE OF SHEEP

Fig 8 —*Cl oedematiens*   Lenticular colony grown in capillary
tube of V.F. agar.   ✗ 100.
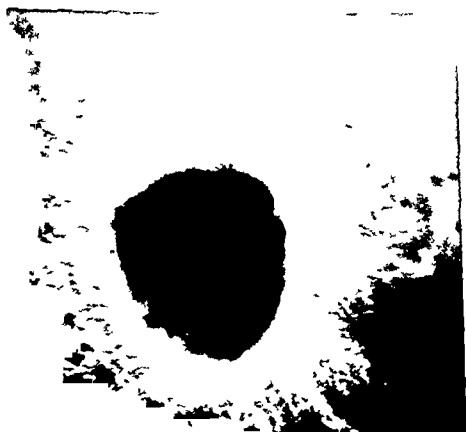
Fig 9 —Later stage than fig 8   The
   en grenade " form   ✗ 100
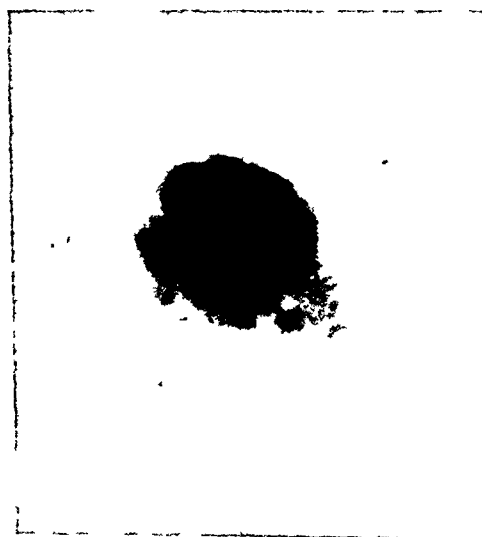
Fig 10 —Surface colony of *Cl oedematiens* on
   V F. agar plate.   This appearance is very
   rare.   ✗ 90.

substantial leucocytic barrier and the greater disintegration of the necrotic cells. Within the leucocytic barrier of the experimental lesions large numbers of vegetative bacilli were seen. The minor differences in the experimental lesion could result from the activation of a larger number of spores than are present in the natural infection, with consequent death of the animals before the defence mechanism had reached the stage usually seen in the natural disease in sheep.

SUMMARY

1. *Clostridium œdematiens* type B is the bacterial cause of black disease of sheep in the North of Scotland.

2. Fermentation reactions cannot be relied upon completely for the identification of *Cl. œdematiens*; but the 17 type-B strains which I isolated from black disease in the sheep failed to ferment glycerol —a finding which supports Turner's results with strains from the same host.

3. Methods of identifying *Cl. œdematiens* types A and B are described and an account is given of tests devised for lecithinase type confirmation. Type-B strains are more fickle in their growth requirements than type-A strains. Consequently many of the tests on the surface of solid media are less reliable for type-B than for type-A strains. A modification of Petrie and Steabben's method proved the most reliable.

4. The production of lecithinase depends upon the medium in which the strains are grown and the limited evidence available indicates that the more rapidly the organism grows the less lecithinase is produced.

5. In black-disease areas 17·1 per cent. of apparently healthy livers from various host species harbour latent spores of *Cl. œdematiens* compared with 1·3 per cent. in healthy livers obtained in areas free from the disease.

6. The pathogenesis of black disease has been shown, by histological evidence obtained from naturally occurring cases of the disease and from experimental evidence in guinea-pigs, to be the activation of latent spores of *Cl. œdematiens* in necrotic foci in the liver caused by the wanderings of the immature liver fluke *Fasciola hepatica*. Attempts to reproduce the disease in rabbits were not successful.

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Appendix
Preparation of Weinberg's V.F. broth and agar

To prepare Weinberg's V.F. broth and agar a stock solution is made up as follows. Fresh ox liver 5·5 lb., fresh ox muscle 5·5 lb. and fresh pig stomach 7 lb. are each minced finely and placed in a large earthenware vessel. Twenty-five litres of tap water and 250 ml. of pure concentrated HCL are now added and the mixture is kept at 48-50° C. for 24 hours. The temperature is then raised to 80° C. to destroy the pepsin and the mixture allowed to stand for a further 24 hours, when it is decanted and filtered through moistened Chardin paper. The filtrate is filled into 500-ml. bottles, steamed at 100° C. for 15 minutes and stored. To prepare V.F. broth the stock solution is heated to 80-90° C., adjusted to pH 8 and heated at 120° C. for 15 minutes to precipitate earthy phosphates. It is then distributed into bottles of convenient size and agar is added in the proportion required, *i.e.* 0·5 per cent. for capillary-tube cultures and 2·5 per cent. for plate cultures.

If properly prepared the stock solution keeps for as long as nine months. Culture forms of *Cl. œdematiens* in this medium are shown in figs. 8-10.

HISTOLOGICAL OBSERVATIONS ON DERMATOMYOSITIS

WALTER PAGEL, A. L. WOOLF and R. ASHER

From the Central Middlesex Hospital, London

(PLATES XC-XCVII)

THE skin and muscle changes from which dermatomyositis derives its name appear to be a mask beneath which equally fundamental changes may be taking place in all parts of the body. Fahr (1921) has laid emphasis on a general involvement of the vascular system as the underlying cause of the muscular and dermal changes in the case which he reports. Dermatomyositis sometimes resembles clinically another skin disease in which systemic manifestations have been discovered, namely lupus erythematosus disseminatus (Keil, 1940 ; Klemperer *et al.*, 1941 ; Banks, 1941). In this disease a generalised degeneration of the collagenous tissue has been suggested as the primary change (Klemperer *et al.*). Does the same apply to the recognised manifestations of dermatomyositis, including the changes in the vessel walls ? The following material has been examined particularly from this point of view.

CASES OF DERMATOMYOSITIS

Case I

Clinical history. The patient, a boy aged 13, was admitted on 24th August 1943 with a history of frequent sore throats. Eight months previously he had had puffiness and discolouration around the eyes and, four weeks before admission, a very sore throat, general malaise and slight pains in the elbows. Regurgitation of fluids and muscular weakness followed. *2nd Sept.* All attempts at detecting the presence of *Str. hœmolyticus* in the throat, including estimations of the anti-streptolysin titre, were unsuccessful : elbows and knees painful. *4th Sept.* Shoulders became painful. *9th Sept.* Tachycardia and dyspnœa ; several joints swollen ; pitting œdema of right leg and thigh ; serum albumin 3·8 per cent., globulin 1·2 per cent.

Biopsy histology, 24th Sept. Muscle biopsy from lateral aspect of thigh. The changes seen (figs. 1 and 2) include (1) waviness of muscle fibres, (2) hyalinisation of muscle fibres with loss of transverse striation, (3) swelling and disintegration of muscle fibres with vacuolation, (4) empty sarcolemmal sheaths and (5) increase in number of nuclei. The latter, which average 12 μ in diameter, are oval and hypochromic, with a distinct basophil nucleolus. They are surrounded by fragments of muscle substance, some of which contain up to

twelve nuclei, thus forming a syncytium. Mitotic figures are scanty, but one or two are seen in each section. Some nuclei are pyknotic and devoid of a nucleolus.

Clinical history (cont.). 25th Sept. Improving ; less œdema. *7th Oct.* Return of œdema. *15th Oct.* Nail-bed telangiectases and purple scars over terminal interphalangeal joints ; B.P. 120/80. *19th Oct.* Two purple patches half-an-inch in diameter on the inner side of the left upper arm. Pink papules on trunk and abdomen. Intermittent bouts of pyrexia.

Necropsy 1943/354. 7th Nov. Gross œdema of muscle septa of arm and thigh. Muscles, especially of thighs and arms, much discoloured, pale and very œdematous. Lymph glands in groin, axilla and antecubital fossa pink and of hazel-nut size. No appreciable visceral changes.

Post-mortem histology. *Biceps femoris.* Three changes are present. (1) Degenerative changes, with muscle fibres appearing hyaline. With Mallory, the muscle substance usually stains red but occasionally mauve or yellow. Some hyaline fibres show retraction clots (Speidel, 1937-38), (fig. 3). With Sudan, fine orange droplets appear beneath the sheath. The intermuscular connective tissue is œdematous and the fibres are broken up into fragments of varying thickness. Where the fragments are particularly thick and straight, they stain yellow to red with Mallory and appear highly refractile. Occasionally muscle is replaced by connective tissue, presumably freshly formed, which has also undergone this change. (2) Proliferation of nuclei with disappearance of muscle substance, leaving the thickened muscle sheaths empty. (3) Vascular changes. These affect many pre-capillary arterioles and consist of eccentric (fig. 4) or concentric, smudgy, intensely eosinophilic swelling of the intima. Sometimes superadded thrombosis occludes the lumen and large numbers of red blood cells are seen in the surrounding muscle. The thickened intima stains red with Mallory. Other arterioles show fibrous intimal hyperplasia or perivascular lymphocytic infiltration. Numerous sections from other skeletal muscles show similar changes. *Skin of eyelid.* Smoothing out of the papillæ due to œdema of sub-papillary connective tissue. Collagen of dermis stains yellow-brown with Mallory and fibres are straightened out, highly refractile and broken up. *Heart.* Great œdema and moderate lymphocytosis of visceral pericardium. Myocardium shows occasional triangular scarring and hyalinisation of muscle bundles. The scars show conglutination of fibres and stain red with Mallory. *Œsophagus.* Muscular coat shows shrinkage of contents of muscle sheaths, with occasional replacement by hyaline masses. *Tonsils.* Retrotonsillar tissue shows thickening and increased eosinophilic staining of collagen. With lowered condenser, collagenous fibres appears as short, thick, highly refractile shreds. *Tongue.* Intense œdema (fig. 5) and " caking " (conglutination of collagenous tissue) of submucosa, which appears intensely eosinophilic. With Mallory, areas are seen where collagenous fibres appear fused into a pale pink material in which it is impossible to distinguish fibre from ground substance. *Peritoneum* (over diaphragm). Severe " caking " of subendothelial collagenous tissue, which stains intensely eosinophilic. With Mallory, there is complete fusion of fibres into red highly refractile bars in which the distinction between fibre and ground substance is no longer possible. *Pleura.* As peritoneum, only less severe. *Sciatic nerve.* Perineural connective tissue " caked " and showing up with Mallory as bright red, highly refractile shreds. *Inferior gluteal artery.* Adventitial collagenous fibres swollen, interrupted and deeply eosinophilic. *Lungs.* Extensive patchy broncho-pneumonic collapse, with leucocytic exudate and coccal colonies. Purulent bronchitis and emphysema. *Kidneys.* Conglutination of collagenous fibres in the adventitia of the interlobar arteries. Glomeruli slightly congested, otherwise no change. *Spleen.* Some arterioles show a localised, deeply eosinophilic, smudgy appearance of the wall, which is swollen at this point and stains

DERMATOMYOSITIS
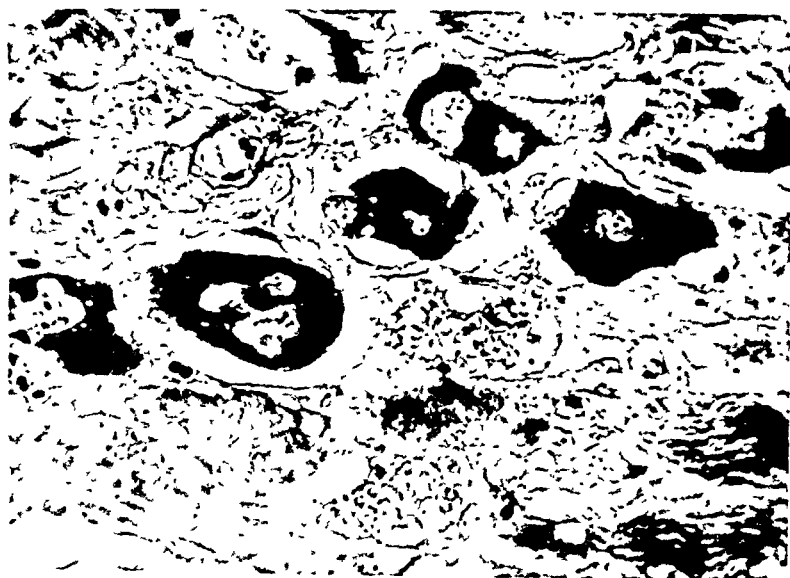
FIG. 1.—Case I. Muscle from lateral aspect of thigh, showing shrinking and vacuolation of muscle substance. Some sheaths appear empty. Haematoxylin and eosin. ×360.
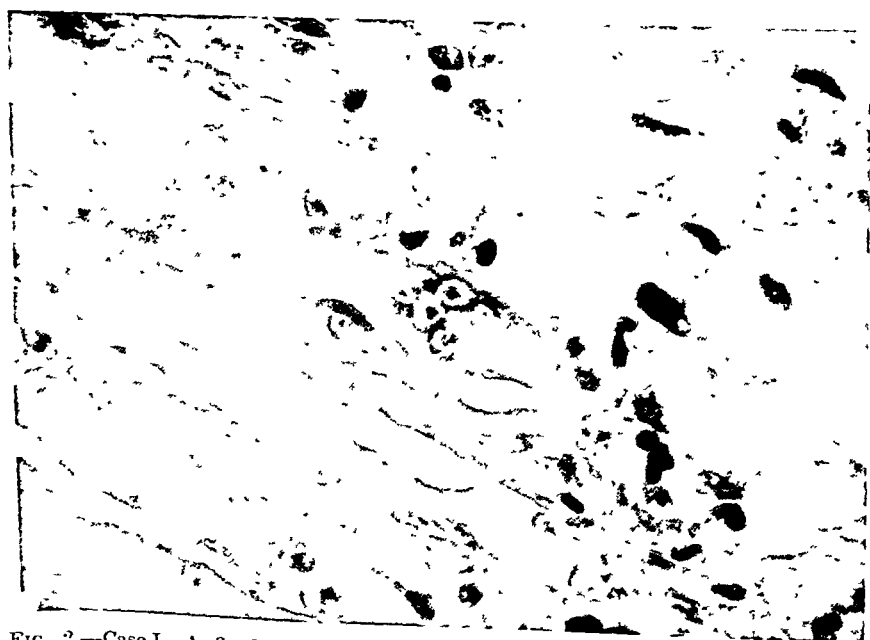
FIG 2.—Case I. As fig. 1, showing sarcolytic muscle bundles with formation of syncytia and longitudinal alignment of hypochromic nuclei. H. and E. ×650.

brilliantly red with Mallory. The thickened capsule when stained in the same way shows conglutination of the collagenous fibres, with formation of innumerable red, highly refractile shreds. *Lymph gland.* A small lymph node is surrounded by smudgy eosinophilic collagenous tissue which is infiltrated by lymphocytes, plasma cells and fibroblasts. *Bone marrow.* Largely aplastic fatty marrow. A few normoblasts and myeloblasts present, with hæmosiderin scattered along small blood vessels. A delicate pale pink reticulum separates the fat globules.

Case II

Clinical history. The patient, a woman aged 63, was admitted on 26th September 1945 with a history of swelling of the wrists on washing for six months. In August there was puffiness of the face and tautness of the facial muscles, followed by loss of power, mainly in the right shoulder. She had noticed that her voice was nasal and that fluids regurgitated through her nose. There was diarrhœa at the onset. On examination, puffiness of the infra-orbital region ; scaly dry skin on back of neck, front of chest and forearms ; brawny swelling of arms and slight non-pitting swelling of face ; dermatographia, greatest between shoulders ; hands blue and mottled ; B.P. 190/90 ; all tendon reflexes absent. Death, which was preceded by fever, occurred on 10th Oct. 1945.

Biopsy histology, 26th Sept. Muscle biopsy from right pectoralis major shows (1) hyalinisation and (2) shrinking of muscle substance with loss of nuclei, (3) empty sheaths, (4) intensely eosinophilic thickening of the walls of small arteries, narrowing (almost obliterating) the lumen, and (5) great œdema and congestion of the overlying dermis.

Necropsy 1945/285. 22nd Oct. Obesity. Muscles in general pale pink. Much œdema of skin and muscles. No appreciable visceral changes.

Post-mortem histology. Skin from eyelid. Œdema of dermis and flattening of papillæ (fig. 6). *Muscle.* Numerous sections were made from various skeletal muscles and from the œsophagus, pharynx and retro-tonsillar region. Three groups of changes are present. (1) Degenerative changes : (*a*) loss of transverse striation due to hyalinisation of the muscle fibres, (*b*) variation in staining affinity of the muscle substance—gradation of colour (with Mallory) from red, via orange and bright yellow, to mauve, (*c*) empty sheaths, (*d*) an occasional muscle fibre showing formation of transverse bands (fig. 7). (2) Collections of cells ranging from simple polyhedral elements to syncytia with oval hypochromic nuclei. (3) Vascular changes, comprising concentric (fig. 8) or eccentric eosinophilic thickening of the intima of precapillary arterioles, sometimes with complete occlusion ; perivascular hæmorrhages present in perimysium. *Tongue.* Extreme " caking " and œdema of subpapillary connective tissue. With Mallory, some collagenous fibres are seen to be fused into red, highly refractile, homogeneous masses. *Retro-tonsillar region.* Between the muscle bundles there is one area consisting of " fibrinoid " eosinophilic material (fig. 9). The adjacent muscle bundles merge gradually with this mesh work, first appearing waxy and thin and fusing across their sheaths with other bundles. In sections stained with Mallory the greater part of the mesh stains blue but in places the fibres are red and fused together into a homogeneous mass. *Myocardium.* Around the blood vessels there is frequently a delicate, filmy eosinophilic mesh staining blue with Mallory. *Kidneys.* Hyaline casts in proximal convoluted tubules. Focal œdema in subcortical renal tissue, with the same eosinophilic change in the walls of the interlobular and arcuate arteries as described in the vessels of the muscles. A few hyaline glomeruli in œdematous areas in the juxta-medullary region. *Spleen.* Severe eosinophilic thickening of the walls of the pre-capillary arterioles, with narrowing and sometimes occlusion of the lumen. With Mallory, the arteriolar walls stain red and are highly refractile.

Case III

Clinical history. The patient, a married woman aged 32, was admitted on 1st Jan. 1948, with an indefinite history of rheumatism in childhood. She developed dermatomyositis after a stillbirth in 1940. Ulceration of the lesions commenced in 1942. Striking features were the ulceration of sclerodermatous patches (fig. 10), tapering fingers, complete ankylosis of the knee joints (high amputation of both lower limbs, 26th April 1948), extreme wasting of muscles and scarring, with apparent lymphatic obstruction, in breasts. The ulceration appeared to improve in hospital with vitamin D and calcium administration. B.P. 136/70. Serum Wassermann reaction negative. After the double amputation, the patient's general condition has remained satisfactory up to the time of writing (September 1949) and there has been no recurrence except some slight and transient blistering, followed by ulceration, of the skin of the breasts and thigh stumps.

Examination of amputated legs. These were extremely wasted and showed ankylosis of both knee joints. Below the junction of the middle and upper thirds of the thigh, there was no normal-looking skin on either leg, except that of the dorsum of the toes and soles of the feet. The remainder of the skin was livid, atrophic and adherent to the underlying tissue, and was the seat of irregularly raised nodules, many of which had ulcerated, leaving approximately circular ulcers with rounded edges, shallow walls and a smooth, dark red or purulent base (fig. 10). The muscles were of normal colour and firm consistency and showed no naked-eye changes.

Histology. Skin. The cutis is extremely atrophic, with ironing out of the papillary processes and a thin but definite rim of keratinisation. There is gross thickening, œdema and " caking " of the collagenous tissue of the dermis, especially in the deeper layers, which are infiltrated by small and large lymphocytes, plasma cells, histiocytes and a few eosinophils (figs. 11 and 12). In the deeper part of these sections there is fragmentation of muscle fibres, with marked lymphorrhagia (perivascular accumulation of lymphocytes, fig. 11). *Muscle.* Sections from seven muscles were examined. There is thickening and " caking " of the collagenous fibres of the perimysium, with lymphocytic and histiocytic infiltration. Within the muscle bundles there are focal collections of hyperchromic nuclei similar to some of those observed in case I, apparently replacing degenerated muscle (fig. 13). The larger arteries (posterior tibial) show great intimal thickening and perivascular lymphocytic infiltration of the vasa vasorum. This infiltration can also be seen in the smaller vessels, where it occasionally forms intimal cushions (fig. 14). In the smaller arteries there is severe œdema, with thickening of the whole wall due to the effusion of an intensely eosinophilic structureless material.

DISCUSSION

These three cases show the clinical as well as the anatomical hallmarks of dermatomyositis, and are in this respect similar to those described in the literature (Parkes Weber and Gray, 1924 ; Ingram and Stewart, 1934 ; Keil, 1940). For discussion, the following points are selected :—(1) The sore throat. (2) The changes in the muscles. (3) The changes in the vessels. (4) The changes in the connective tissue.

1. *The sore throat*

Sore throat has preceded the onset of dermatomyositis in several recorded cases (Koster, 1897 ; Fahr, 1921 ; Clark, 1946). In the

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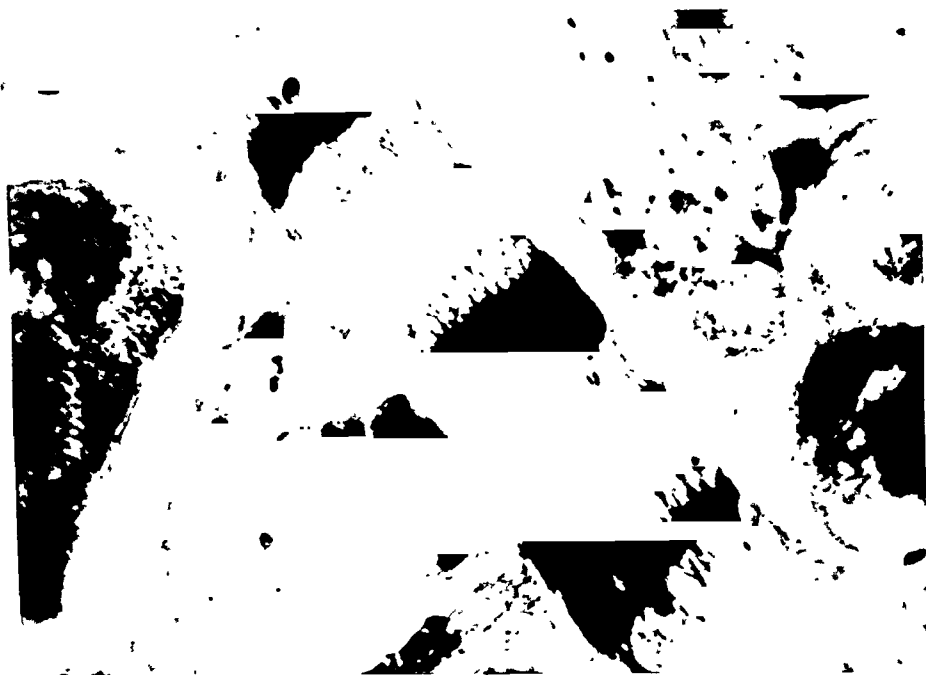
FIG. 3.—Case I. Biceps femoris, showing retraction clots in hyaline muscle fibre. Mallory. ×850.
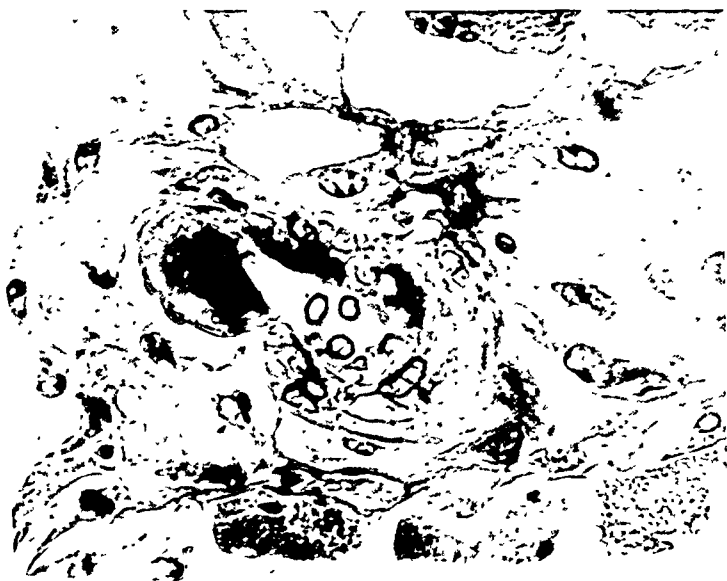
FIG. 4.—Case I. As fig. 3, showing œdema and degeneration of muscle bundles with intensely eosinophilic (fibrinoid) swelling of wall of central arteriole. H. and E. ×400.

PLATE XCII

DERMATOMYOSITIS
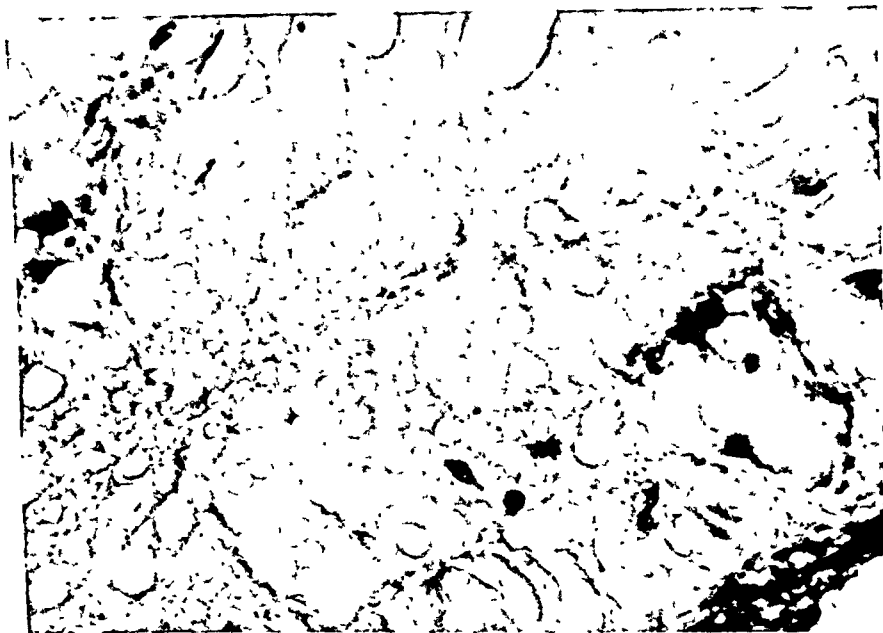
FIG. 5.—Case I. Tongue, showing gross œdema of submucous connective tissue. H. and E. ×360.

FIG. 6.—Case II. Skin, showing " ironing out " of epidermal papillæ and gross œdema of subepidermal connective tissue. H. and E. ×160.
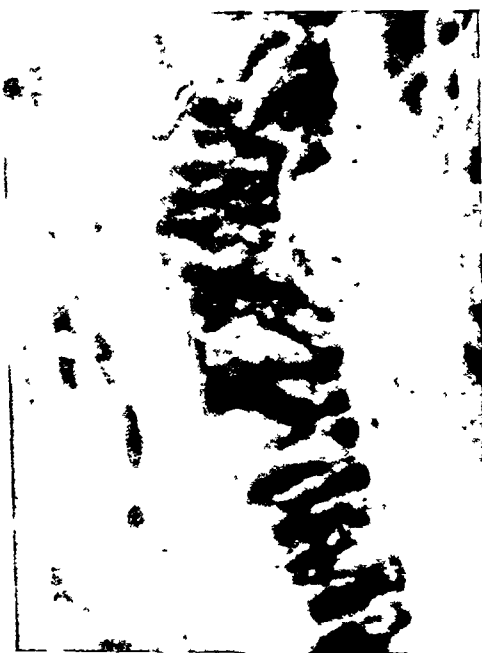
FIG. 7.—Case II. Rectus femoris, showing transverse bands in swollen muscle fibre. Mallory. ×700.

DERMATOMYOSITIS

FIG. 8.—Case II. Rectus femoris, showing eosinophilic fibrinoid swelling of wall of pre-capillary arteriole in perimuscular connective tissue, with surrounding œdema. H. and E. ×450.
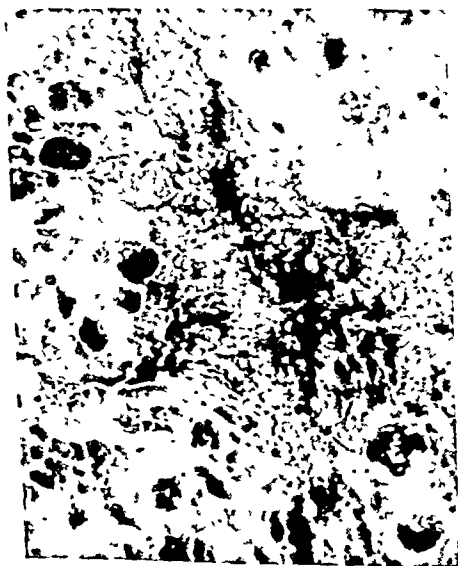
FIG. 9.—Case II. Retro-tonsillar tissue, showing formation of eosinophilic fibrinoid material between necrotic bundles of pharyngeal muscle. H. and E. ×350.

FIG. 10.—Case III. Right lower limb, showing ulcers, nodules and atrophic skin.

FIG. 11.—Case III. Deep connective tissue of skin, showing œdema, " caking " of collagenous fibres and transverse rows of lymphocytes, plasma cells and histiocytes. There are also perivascular collections of lymphocytes (lymphorrhages). H. and E. ×100.

FIG. 12.—Case III. As fig. 11, but higher magnification, showing rows of lymphocytes, plasma cells and histiocytes. H. and E. ×500.

FIG. 13.—Case III. Skeletal muscle, showing island of myogenic regenerative cells. H. and E. ×450.

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FIG. 14.—Case III. Branch of posterior tibial artery with partly hyalinised wall and sub-intimal collection of lymphocytes surrounding a capillary and bulging into the arterial lumen. H. and E. ×120.
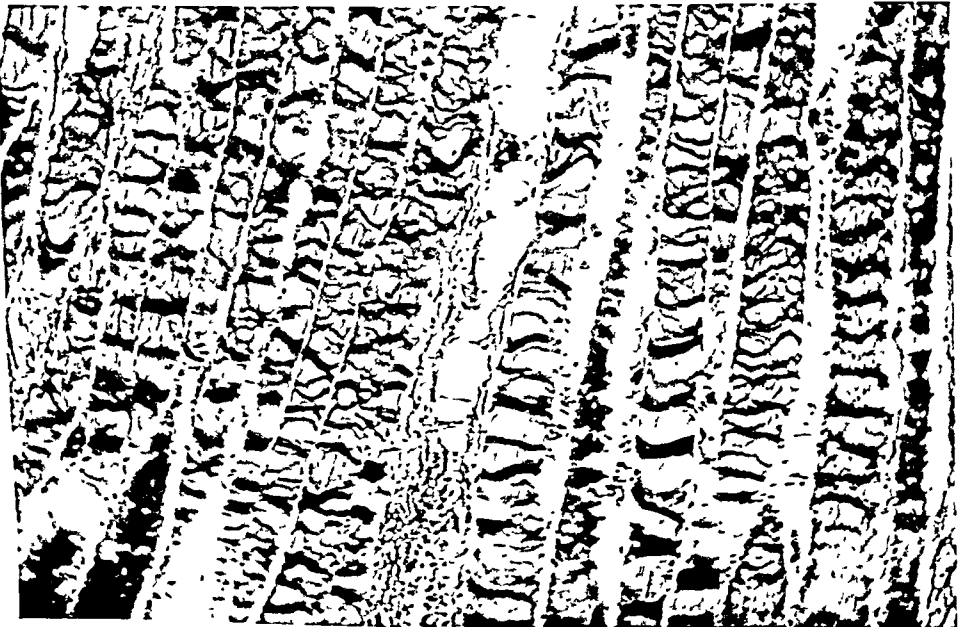
FIG. 15.—Traumatic degeneration of muscle in a woman aged 32. Latissimus dorsi, showing prominent band formation. Mallory. ×120.

DERMATOMYOSITIS

FIG. 16.—As fig. 15, but higher magnification, showing persistence of transverse striation between the bands. Mallory.   ×600.

FIG. 17.—Same case as in figs. 15 and 16. Transverse section, showing waxy degeneration and vacuolation. H. and E.   ×180.

FIG. 18.—" Myositis of unknown origin " in a woman of 60. Sacrospinalis showing increase in nuclei and a syncytium.   H. and E.   ×520.

present case I, sore throat was not only a striking event in the immediate history, but had occurred previously on many occasions. In case II, in spite of the absence of symptoms referable to the locality, severe changes were present in the muscles and vessels of the tonsillar region and in the pharyngeal muscles. There was no symptom suggestive of pharyngeal sepsis in the history of case III.

2. *The changes in the muscles*

These consist of a profound dissolution of the striated muscle fibres and various other cellular changes. Among the cells appearing in relation to the disintegrating muscle, the syncytial elements would seem to be formed from fragments of muscle substance. The other cells resemble, in appearance and situation, those emerging from disintegrating muscle during amphibian metamorphosis (Glücksmann, 1934). The question arises as to how far these changes constitute an entity typical of or even specific for dermatomyositis. This theory we are not inclined to accept, for all these changes can be seen, for example, in trauma. The transverse band formation, a prominent feature in case II (fig. 7), was the most striking change (figs. 15 and 16) in a section through a swelling in the latissimus dorsi that followed trauma in a woman aged 32 (surgical biopsy no. 3112). The band formation also appears to be identical with that described by Schmidt (1910) in injury due to electrocution and is one of a whole series of bizarre alterations in the arrangement of the contractile substance which have been observed in man under the most varied conditions affecting the vitality of the muscle (Zenker, 1864) and can be produced experimentally by a variety of noxious agents (Thoma, 1906 ; Speidel, 1939), including in all probability operative trauma. Disintegrating muscle surrounded by cells of characteristic appearance is a common feature in biopsies from cases of " myositis of unknown origin." This is shown in fig. 18. It demonstrates a section through a swelling of the sacrospinalis which had first been observed some three months before by a woman aged 60 and had been preceded by three years of pain in the back (surgical biopsy no. 5032). The most impressive change in this case was clustering of nuclei and syncytia around degenerate muscle substance, apparently as a prelude to repair and regeneration. This is borne out by the findings of Le Gros Clark and Blomfield (1945), in which muscle regeneration was shown to commence with the formation of syncytia.

3. *The vascular changes*

Vascular changes were present in all three cases of dermatomyositis. The most characteristic finding was concentric and eccentric, sub-endothelial, homogeneous, intensely eosinophilic fibrinoid thickening of the walls of arterioles, some of which were thereby occluded ;

nuclei were absent or pyknotic in the thickened areas. Perivascular hæmorrhage, lymphocytic infiltration and œdema were also prominent. It was noted that these changes were often present in the centre of an area of necrotic muscle. The nature of the change in the vessel wall is obscure. Although the thickened segment of the wall resembled fibrin in its affinity for eosin and acid fuchsin (with Mallory), it differed from it in its pale staining with aniline methyl violet and Mallory's phosphotungstic acid-hæmatoxylin. These changes were observed by Fahr in a case of dermatomyositis and compared by him with those seen in malignant nephrosclerosis and in some cases of rheumatic myocarditis. There was no hypertension in case I of the present series, nor was there, in any of the three present cases, the hyalinisation of the vascular circumference which has been regarded as typical of malignant hypertension (Klemperer *et al.*). Moreover, the arteriolar changes were restricted to muscle and skin and absent from the sites of predilection for hypertensive arteriolar changes— kidney, spleen, pancreas, etc. Morphologically, the arteriolar changes resembled those observed in various conditions other than malignant hypertension, for example subacute bacterial endocarditis. In one such case, a woman aged 23 (necropsy 1944/103), we observed in the tongue (fig. 19), myocardium and diaphragmatic pleura fibrinoid thickening of the intima of the arterioles in one part of the circumference. In some arterioles the whole lumen was filled by a hyaline patch with only a few capillary slits. Associated with and extending out from these vascular changes were œdema, hyaline swelling and homogenisation of collagenous tissue, which stained orange with Mallory and Van Gieson. In other words we see here the same combination of vascular and collagenous fibre changes as in dermatomyositis. In a further case, a woman aged 50 (necropsy 1946/270), with a history of Raynaud's syndrome for twenty years and presenting with rheumatoid arthritis and scleroderma with impending gangrene of the fingers, œdema together with similar arteriolar changes was present in the œsophagus, pancreas, thyroid, stomach and breast. It is notworthy that in this instance the changes in the vessels were associated with " scleroderma," that is, skin lesions (fig. 20) similar to those found in dermatomyositis. In yet another case extensive arteriolar changes (fig. 21) of the sort described in our dermatomyositis cases occurred with fulminant tuberculous septicæmia (Blair and Pagel, 1947).

The changes in the vessels in dermatomyositis, therefore, do not appear to be specific for this disease, since they occur also in a variety of conditions in which prior damage to the vessel wall may be assumed.

4. *Changes in the connective tissue*

In all our three cases of dermatomyositis, changes were present in the connective tissue, but their full extent could only be assessed

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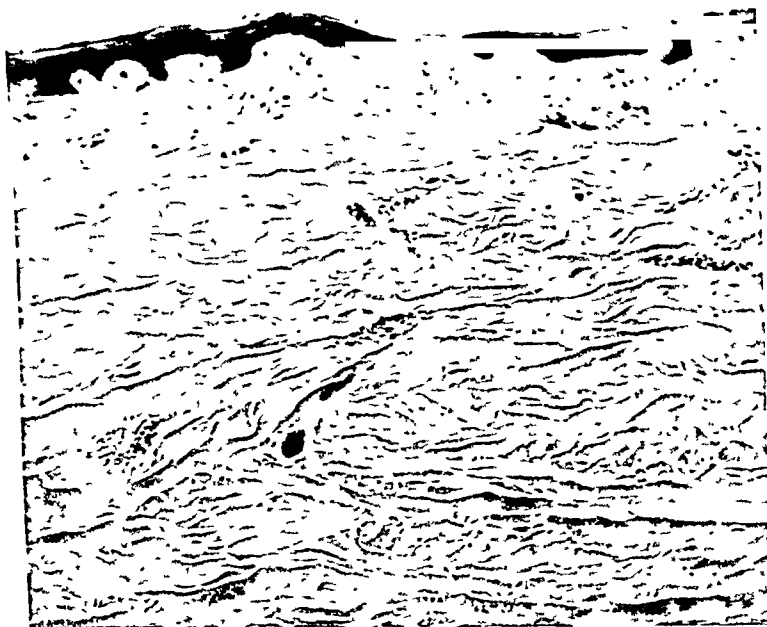
FIG 20 —Scleroderma in a woman aged 50. Skin, showing flattening of papillæ and caking of collagenous fibres. H. and E. ×85.

FIG 19 —Subacute bacterial endocarditis in a woman aged 23. Tongue, showing intensely eosinophilic fibrinoid swelling of capillary wall (arrow) extending into interstitial tissue. H. and E. ×400.

FIG. 21.—Fulminant tuberculous septicæmia in a man aged 54. Myocardium, showing intensely eosinophilic ridge in arteriole H. and E. ×600.

in the first two, which were fatal. Here the collagenous tissue of almost all parts of the body was affected, the retro-tonsillar region, tongue and sub-mesothelial tissue being particularly affected. The earliest and most constant finding was œdema, with formation of a fibrinous or fibrinoid network which was later converted into a filmy collagenous mesh staining blue with Mallory. In more severely affected areas this stain showed " caking " of the collagenous fibres, which lost their previously delicate outline and became swollen and highly refractile, later breaking up into shreds or bars staining red with Mallory. Still later, the fibres fused into a homogeneous mass. Both in quality and distribution, these changes resemble those described by Klemperer *et al.* in lupus erythematosus disseminatus, and a similar observation has been recorded by Bergstrand (1946) in the so-called " allergic syndrome " (*vide infra*).

Klemperer *et al.* suggest that lupus erythematosus disseminatus is a generalised disease of collagen. They draw attention to the similarity to rheumatism, glomerulonephritis, polyarteritis nodosa, malignant hypertension and eclampsia, but are careful to point out important differences. In our opinion, dermatomyositis resembles lupus erythematosus disseminatus in being a diffuse disease of collagen throughout the body, but differs from it in the additional involvement of the muscles and possibly in the absence of a high incidence of renal and endocardial lesions. The diffuse change appears to be œdema, the ætiology of which, in view of the frequent history of throat infection, may well be anaphylactic. This is in accord with the well-known observation that swelling (fibrinoid degeneration) of the collagenous fibres, including those of the intima of vessels, is an essential feature of " hyperergic " inflammation—the Arthus phenomenon—(lit. Pagel, 1939).

Baehr and Pollack (1947) reject the presence of fibrinoid change in the collagen as an indication of an " allergic " basis for a disease and, in addition to local fibrinoid necrosis, point to the diffuse occurrence of this change in malignant hypertension. However, while we are still ignorant of possible " allergic " factors in the causation of malignant hypertension (Loomis, 1946), it is the restriction of the fibrinoid change to the vessel wall rather than its independent occurrence in the connective tissue that is typical of malignant hypertension. That local fibrinoid necrosis can occur where rapidity of onset makes sensitisation improbable is shown in thermal injury of the dermis (Moritz, 1947).

Diffuse fibrinoid change in the connective tissue is seen only in lupus erythematosus disseminatus, dermatomyositis and scleroderma —all diseases of unknown ætiology—and in rheumatic fever and polyarteritis nodosa, for which a mechanism of specific hypersensitivity is being increasingly regarded as operative (Rich and Gregory, 1943). Further, Bergstrand (1946) has demonstrated its presence in the " allergic " syndrome of transient lung infiltration, eosinophilia and

urticaria. Finally, a hyperergic myositis has been induced in rabbits by intramuscular injection of a muscular antiserum (Kallós and Pagel, quoted by Pagel, 1939, p. 126).

In conclusion it may be stated that, while none of the morphological changes in dermatomyositis is specific, collectively they do seem to form an entity characterised by a progressive and diffuse affection of skin and striped muscle.

SUMMARY AND CONCLUSIONS

1. Three cases of dermatomyositis are reported which showed a triad of changes affecting striated muscle, blood vessels and connective tissue.

2. The most striking changes in muscle were (a) vacuolation and shrinking of the muscle substance, with altered staining affinity and loss of transverse striation, and (b) increase in the number of myogenic nuclei, which appeared surrounded by fragments of muscle substance so that syncytia were sometimes formed. Comparative studies showed these changes to be non-specific, since they are observed in muscle subjected to various pathogenic stimuli.

3. The principal vascular change was annular or localised fibrinoid degeneration of the arteriolar walls, occasionally leading to occlusion of the lumen followed by hæmorrhage. In two of the cases this occurred in the absence of hypertension.

4. In the connective tissue extensive œdema and fibrinoid change in the collagenous fibres were observed.

5. The non-specific nature of the changes described in (3) and (4) was illustrated by their occurrence in cases of subacute bacterial endocarditis, in scleroderma with rheumatoid arthritis and in fulminant tuberculous septicæmia.

6. The extent to which these changes resemble the " diffuse collagen disease " postulated by Klemperer et al. is discussed.

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616 . 314 . 17—008 . 1—018—02 (*Cricetus auratus*)

HISTOLOGICAL OBSERVATIONS ON PARODONTAL DISEASE IN THE GOLDEN HAMSTER (*CRICETUS AURATUS*): CALCULUS, FOOD PARTICLES AND OTHER FOREIGN BODIES AS ÆTIOLOGICAL FACTORS

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(PLATES XCVIII-CII)

THE clinical and gross post-mortem features of a severe form of parodontal disease in the golden or Syrian hamster have been recently described by King and Gimson (1948-49).

Observations were made on the oral conditions in thirty animals fed from soon after weaning on a daily diet comprising cereal (6-9 g.), separated milk powder (1 g.), food yeast (*Torula utilis* (0·1 g.) and sodium chloride (0·5 g.) per hamster, with additions of lean minced meat (3 g.) and fresh cabbage (3 g.) twice weekly. Three varieties of cereal were used—wholemeal flour, wheatmeal bread (crumb) and coarsely or finely milled white maize. Experimental vitamin supplements were given in the form of β-carotene, vitamin D and cod-liver oil, while controls received vitamin A- and D-free peanut oil. Parodontal lesions, easily visible to the naked eye at autopsy, were found in some degree in three-quarters of the animals after experimental periods of 19-65 weeks, but no significant differences in their incidence or extent were apparent between the main cereal groups or between their respective supplemented sub-groups. No attempt was made to render these animals either rachitic or deficient in vitamin A, such conditions being prevented respectively by the calcium and phosphorus content of the basal diets and by the carotene-containing cabbage, so that the vitamin additions constituted dietary reinforcements of basal rations already adequate in vitamins and mineral salts under ordinary conditions. In order of frequency, the percentage incidence of gross lesions in the various regions of the mouth were :—upper third molar 66·7, upper second molar 36·7, lower first molar 25·0, upper first and lower second molar each 6·7 and lower third molar 1·7. Macroscopically, the disease was characterised by extensive deposits on and between the teeth, with adjacent parodontal involvement. These accretions included food particles and foreign bodies such as wood-shavings, sawdust and hairs. It seemed significant that the initial lesions arose close to the openings of the salivary ducts, a phenomenon analogous to that in ferrets, in which parodontal disease was induced by direct injury of the gingivæ and other structures by calculus (King, 1944, 1945 ; King and Glover, 1945). In the hamster, however, unlike the ferret, the morphology and function of the teeth concerned are such that maxillary labioversion, mandibular linguo-version and torsion of the teeth are common phenomena.

The present communication concerns the histological changes observed in the jaws of the same series of hamsters, with special reference to their ætiology and progress.

The material largely comprised paraffin sections, 7-10 μ in thickness, of decalcified formol-fixed specimens from one side of both jaws of each animal, the maxillary sections being serial; Wittmaack's acetic acid-bichromate-formalin fixative was employed on occasion. Nitric acid (2 per cent.) was used for decalcifying throughout. In two animals serial sections were made of half-jaws with all oral and neighbouring tissues *in situ* for studying the relationship of the molar teeth to the salivary glands and ducts and to the cheek-pouches and other structures. Staining methods included Ehrlich's hæmatoxylin and eosin, Weigert's iron hæmatoxylin and Van Gieson, a Mallory modification, and Gram. For identifying food particles and foreign bodies, alcohol-fixed frozen sections were made and stained by Ehrlich's hæmatoxylin and eosin, with and without previous acid treatment, and by alcoholic purpurin.

The microscopic findings in general confirmed the previous clinical and gross post-mortem observations (King and Gimson, 1948-49) but disclosed a much higher incidence of disease involving all animals of the three series irrespective of their basal diets and experimental supplements. Of the individual molar regions only the distal aspect of the lower third molars and the mesial side of the upper first molars were but little affected over experimental periods of 19-65 weeks.

General course of the lesions

The disease is initiated by deposits of salivary calculus (tartar) upon the crowns of the molar teeth. The primary accretions occur in the deeper parts of those intercuspal grooves where the enamel cuticle is most dense and most persistent and which lie nearest to the openings of the regional salivary ducts. Thus, in the maxilla the labial surfaces of the teeth are first involved, the initial deposits being laid down in the intercuspal grooves of the third molar tooth. Laceration of the adjacent gum margin by the rapidly encroaching tartar with pocket formation soon follows. The consequent exposure of the subgingival epithelium of the pocket wall next provides a suitable stroma or nutrient source for calculus-depositing agencies and, with impaction of food and other debris in the pocket and in the adjacent interdental gum anteriorly, tartar soon forms on the mesial aspect of the third and distal aspect of the second molars. At a later stage the labial intercuspal grooves and then the mesial aspect of the second molar are similarly involved. Finally, but much later, the process extends to the mesial side of the first molar in spite of its relative remoteness from the parotid or other salivary ducts. The palatal sides of these teeth are affected in the same sequence,

but apart from the third molar, whose palatal side is not far distant from the infra-orbital ducts, calculus deposits in these areas are more dependent on extension of gum lesions from the labial and mesial aspects, on impaction of other foreign bodies and on the resulting degree of labioversion of the teeth, as discussed later. The primary site of tartar formation in the mandible is the lingual intercuspal groove of the first molar, in proximity to the salivary ducts opening nearby. Following a similar process to that in the maxilla, but in a reverse direction, tartar is laid down on the various surfaces of the second and finally the third molars, aided again by extension of gum pocketing, impaction of debris and, in this instance, by linguoversion of the teeth. As pointed out in the earlier paper (King and Gimson, 1948-49), the labial displacement of the upper teeth and the lingual movement of the lower are themselves accentuated by the stresses of dental occlusion and mastication. There is also a natural lateral inclination of the molar teeth—labially in the maxilla and lingually in the mandible—which renders them particularly susceptible to pathological displacement in these directions.

Histopathology

The first parodontal structure to become injured by the progress of calculus along the enamel cuticle is the keratinous layer of the gingival crest at its point of apposition to the cuticle. In this region the remnants of the enamel organ, which comprise the subgingival epithelium after tooth eruption, are in organic continuity on the one side with that part of the enamel organ now forming the enamel cuticle (Nasmyth's membrane) and on the other with the gum epithelium. Contrary to the contentions of most dental histologists, therefore, the keratin-like enamel cuticle and the keratinous layer of the gum are merely in apposition externally. Since the cuticle is irreplaceable, while the gum surface is constantly being cast off and replaced from below, connection between the two can only be brought about by the continuity of the respective cellular elements of the epithelium from which they originate. There is in fact a potential " gap " or sulcus between tooth and gingival crest, of which accumulating calculus and impacting food and other debris take full advantage.

Early disintegration of the keratinous layer of the gingival crest, with defensive proliferation of the subjacent epithelium, appears to be common to all species susceptible to salivary calculus, but the next phase in the hamster, while simulating lesions in the human upper first molar region, differs fundamentally from that in the ferret's carnassial and human incisor areas (King, 1944). For example, in the upper jaw the ferret disease, following marked proliferation of the gingival epithelium into the infiltrated corium, is characterised by eversion of the labial gingivæ and progressive destruction of the keratinous layer of this epithelium from crest to labial sulcus ; the

subgingival epithelium is not involved in the earlier phases. In the hamster, much less of the keratinous layer of the gum epithelium is at first destroyed—indeed some thickening of this structure may occur at a little distance from the initial lesion—and the gingival epithelium proliferates to a lesser extent. Eversion of the gum is not prominent and the most severe reactions are found in and adjacent to the subgingival epithelium. Here hyperplasia, keratinisation on exposure to the mouth and degeneration of this epithelium, with associated cellular infiltration of the neighbouring corium, are characteristic features. All of these conditions contribute to rapid deepening of the parodontal pockets, which in turn facilitates further deposition of calculus and impaction of debris. In previous experimental studies on ferrets the writer has suggested that persistence of the enamel cuticle on areas of the tooth crowns close to the openings of the salivary ducts might well be one of the prerequisites for tartar deposition in animals and man (King, 1945). A number of the earlier phenomena of the hamster disease so far encountered provides factual support for a theory of this kind, but the thesis may now be carried further.

On reaching the hamster's gum margin, the calculus causes detachment of the latter from the tooth in the region of the gap or sulcus, to which reference has already been made. This detachment, from which the parodontal pocket originates, exposes a short length of the coronal part of the subgingival epithelium. The latter promptly reacts to such pathological exposure by becoming keratinised and assumes the form and functions of enamel cuticle, a process similar in effect to the physiological development of the original cuticle during tooth eruption. More calculus is then laid down in and around this newly keratinous tissue, which in turn causes exposure of another length of subgingival epithelium for keratinisation and the whole process is repeated at progressively deeper levels until the enamel-cementum junction is reached. With each new peripheral involvement of subgingival epithelium, deeper portions of the latter proliferate into the adjacent corium, in which inflammatory cells appear in varying numbers. As the calculus-induced inflammatory process extends deeper, necrosis and eventual disappearance of the previously attacked superficial tissue occurs—an attempt on the part of the organism to eliminate the parodontal pocket and reduce its liability to the entry of food and other debris. In the hamster, however, the physical nature of the diet and the cage-bedding on which the animals are kept also play important contributory parts. When the diet is finely comminuted, food debris packs into the parodontal pockets initiated by the calculus. In other cases the food particles may themselves be coarse and sharp enough to become impacted in the gum tissues of the pocket walls, and a similar path is followed by sawdust particles and wood-shavings when these materials comprise the bedding of the animals' boxes or cages.

Up to this point the lesions described may apply, with few and minor reservations, to the parodontal tissues around both the labial aspect of the upper third molar and the lingual side of the lower first molar teeth. Their extension, mesially in the maxilla and distally in the mandible, soon follows, the interdental impaction of food and other debris here being a particularly active factor in the spread of disease to the approximal and labial areas of the upper second molar and to the approximal and lingual tissues of the lower second molar regions. About the same time, or sometimes a little earlier in the maxilla, the upper third molar palatal and the lower first molar labial parodontal structures are affected. As these areas become involved it is less easy to distinguish primary from secondary ætiological agencies. It may be that food debris and foreign-body impaction now tend to assume the ascendancy, since they are increasingly evident and the salivary duct orifices gradually become more remote as the lesions proceed anteriorly in the maxilla and posteriorly in the mandible. Certainly appreciable amounts of calcified material are still apparent in the diseased tissues, but some at least of this mineralised debris may be derived from foci of calcification in or around food or other particles which have themselves aroused the initial traumatic inflammatory lesion in the neighbouring or more superficial areas.

The next stage in the upper third molar region includes the changes consequent upon extension of the inflammatory process beyond the enamel-cementum junction, in which the periodontal membrane, the alveolar bone and the tissues of the tooth-root are concerned. As the calculus approaches the enamel-cementum junction on the labial aspect of the teeth, the resulting inflammatory reactions just ahead, with their accompanying proliferation of the terminal part of the subgingival epithelium, encroach upon the upper portion of the periodontal membrane and on the connective tissue covering the alveolar crest, including the various dental suspensory ligaments. Further deposits of calculus then form upon the outer surface of the cementum and among the adjacent inflammatory cells of the affected periodontal membrane. About the same time the deeper vessels of the periodontal membrane become much dilated and osteoclast-like cells appear on the superficial and inner aspects of the alveolar crest. Rarefaction of the latter soon follows, this being associated not only with giant-cell activity but also apparently with pressure resorption due to the gross dilatation of the periodontal and other neighbouring vessels. Calculus accretions continue to progress along the cementum and periodontal membrane and each new deposit arouses first periodontitis and then osteoclasis at successively deeper levels. In this manner progressively increasing alveolar rarefaction follows, with correspondingly increased loss of dental support from the external alveolar plate. Eventually the tooth begins to tilt labially, the ensuing dental movement in turn causing irritation of the whole periodontal

membrane and its attachments from just beyond the zone of labial calculus formation to the dental apex in palatal and approximal as well as labial directions. The most striking reaction to this labioversion is then seen on the palatal aspect of the same upper third molar tooth, in the form of reciprocal bone deposition on the dental side of the palatal alveolus. This reaction, together with one of a similar nature but lesser degree on the outer (labial) aspect of the external alveolar plate, would seem to comprise a natural defence mechanism which prevents loosening of the tooth; on occasion the same defensive measures may be observed approximally in an attempt to check loosening of the tooth from rotation. However, since the disease process continues to cause resorption of the inner side and soon overcomes attempted bone deposition on the outer aspect of the labial alveolus, the more the compensatory palatal bone deposition the more the tooth is tilted towards the cheek.

A vicious circle is thus established, the ill effects of which are still further accentuated during occlusion by the impact of the affected tooth with its as yet relatively disease-free mandibular opponent; this additional labially-directed stress is itself assisted by the inherent inclination of the upper teeth towards the cheek. Moreover, although calculus may be less extensive on the palatal aspect of the tooth in question, it is nevertheless a pathogenic agency which in this area is soon assisted by impaction of food particles and other debris. At the same time extension of the lesions from both labial and palatal directions causes additional weakening of the dental suspensory apparatus. In the later phases of labioversion the occlusal part of the tooth crown lies in direct apposition to the inner aspect of the cheek and may become entirely covered by a dense mass of calculus and debris, contact with which may even cause small patches of necrosis in the buccal mucosa. Finally destruction of the parodontal tissues proceeds to such a degree that the tooth itself becomes exfoliated.

The foregoing account of the later sequelæ of the hamster disease applies more particularly to the upper third molar regions where the lesions first become prominent. Many of the events described also hold good for the lower first molar areas, where the disease has its mandibular origin, but here allowance has to be made for the lingual position of the salivary duct orifices and the natural inclination of the mandibular teeth towards the tongue. In this instance, therefore, the progress of calculus formation and debris impaction is greater on the lingual aspect of the tooth. The resulting pathological changes thus include rarefaction of the internal alveolar plate, with attempted compensatory bone deposition on the inner side of the external plate, extension of the disease from the first molar posteriorly via its distal aspect, linguoversion of the tooth accentuated by occlusion with its relatively disease-free maxillary opponent, and eventually exfoliation in a lingual direction.

As regards extension of the disease to other regions, the brief account already given of the manner in which the upper second molar tissues become involved is applicable at a later stage to the first maxillary molar region. In the mandible, too, spread of the lesions to the second and finally the third molar region is modified only to conform with their lingual and linguo-approximal origin, although in both jaws the ætiology of the earlier and intermediate disease phases in these areas may perhaps be more closely associated with traumatism of the gum by food or foreign body impaction.

Reasons of space economy preclude detailed illustration of all the disease phases so far described but some characteristic changes in the maxilla may be shown (figs. 1-13).

Fig. 1 demonstrates the relative extent of lesions in the three upper molar regions of one side of a hamster following experimental feeding for 23 weeks. The disease is here fairly advanced and the initial phases are now obscured by its spread anteriorly. The mesial tissues of the first molar region, however, are not yet affected to any marked extent. The difference in severity of calculus formation and gingival disease on the labial and palatal aspects of the upper third molar is shown in fig. 2. Conditions to be noted in this specimen include, labially, massive calculus formation with close adherence to the enamel cuticle, causing deep gingival pocketing, thickening of the keratinous layer of the gingival and buccal epithelium, marked cellular infiltration of the adjacent gum corium and apposition of the coronal calculus to the cheek arousing thickening of the buccal epithelium due to labioversion of the tooth ; palatally, there is thickening of the gum epithelium and of its keratinous layer with gross proliferation of the subgingival epithelium, related inflammation of the corium and pocket formation due to calculus in lesser amount but aided by impaction of food debris. Figs. 3 and 4 demonstrate the bony changes associated with labioversion of an upper third molar on its respective palatal and labial sides. Fig. 4 illustrates resorption of the periodontal aspect of the external alveolar plate due to the progress of the calculous disease beyond the enamel-cementum junction. Irregular thickening of the cementum and gross dilatation of the periodontal vessels are also seen. On the palatal side of the tooth (fig. 3) pocket formation is deep but less extensive than labially and deposition of new bone is visible on the periodontal side of the alveolus. These conditions are shown more clearly under higher magnification in figs. 5 and 6, where osteoblast activity and bone deposition are seen palatally (fig. 5) and bone rarefaction labially (fig. 6). In fig. 6 it should be noted that osteoclasis is strongly aided by grossly dilated periodontal membrane vessels, while externally other cells are attempting to lay down bone in a further attempt to resist tooth loosening. In fig. 7 destruction of inter-radicular bone is shown to result from simple extension of the disease between the tooth roots. Figs. 8-13 illustrate the initiation and extension of the disease in the approximal and other areas of the parodontium in relation to the causative calculus and impacted debris. Conditions to be noted include the close adherence to and progress of calculus along the enamel cuticle (fig. 8), along the exposed and keratinised former subgingival epithelium and along the cementum and periodontal membrane, together with the impaction of food particles and wood debris in the resulting parodontal pockets and interdental spaces. In fig. 10 it is of note that calculus is being laid down in the exposed and thickened keratinous layer of the former subgingival epithelium ; in fig. 9 deposition of tartar is seen to have followed excavation of the cementum of the third molar roots ; and in fig. 11 portions of the grossly impacted food and other debris show evidence of calcification. In the latter connection figs. 12

and 13 are of particular interest. These are high-power photomicrographs of food particles from an interdental parodontal pocket. The structures seen, as in the case of the debris in figs. 8-11, were identified by comparison with stained frozen sections of the dietary components and of the sawdust or wood-shavings used as bedding materials. In the present instance both illustrations are of wholemeal flour particles in transverse section. Possible indications of beginning calcification of their central portions are visible in some areas of fig. 12, but the process is better seen in fig. 13. It should be emphasised, however, that in assessing by histological means calcific changes in or around food particles *in situ*, account must be taken of the original calcium content of the foodstuff in question. In the case of bread (fig. 11), wholemeal flour (figs. 12 and 13) and cabbage (fig. 14), therefore, evidence of calcification is only of pathological significance when the staining reactions indicative of calcification are considerably more intense in the food particles impacted in the parodontal tissues than in control sections of the foodstuffs from which they were derived.

One important feature of the later phases of the hamster syndrome has not yet been mentioned, although a similar phenomenon was previously noted in the ferret (King, 1944) and may have some bearing on the reported production of " dental caries " in hamsters by American workers. Figs. 14 and 15 illustrate later sequelæ and inflammatory changes along the distal root of the second upper molar and adjacent periodontal membrane resulting in excavation of both cementum and dentine of the root. In fig. 14 the cavities have become filled with further deposits of calculus, while proliferation of defensive secondary dentine on the pulp wall is clearly evident opposite the lesions. Incidentally, the secondary dentine is here well calcified, as would be expected in view of the vitamin D and mineral content of this animal's rations (Mellanby, 1922-23, 1930, 1934; Mellanby *et al.*, 1924). Fig. 15 demonstrates a still later phase in which similar cavitation has entirely destroyed a peripheral portion of another maxillary second molar distal root and its protective secondary dentine, with penetration of inflammatory debris and calculus into the pulp cavity. Gross infection of the dental pulp has resulted, followed by secondary infection of the coronal dentine from within. It can be readily seen that superficial examination of such excavated teeth *in situ*, even with the aid of a dissecting microscope or X-rays, might well lead to a false diagnosis of dental caries ; only histological study of serial sections can decide the issue in most cases. Indeed, the writer's present experience of so-called caries in hamsters has led him to view with a certain degree of caution some of the findings of Arnold (1942), Keyes (1944, 1946*a*, *b* and *c*), Keyes and Dale (1944), Dale *et al.* (1944), Sognnaes (1948) and others. All these workers have described the occurrence of two types of carious lesions in hamsters, one originating in the occlusal pits and fissures, the other in the cervico-approximal areas of the molar teeth. As figs. 14 and 15 indicate, some at least of the second type of cavity may be due to parodontal disease rather than dental caries. In the present hamster experiments decalcified serial sections of one side of the

PARODONTAL DISEASE IN HAMSTERS

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

upper jaw of each animal were made. Cavities originating in the root or in the crown were distributed as follows :—

	No. teeth examined in serial section	Teeth with root cavitation of calculous origin (per cent.)	Teeth with crown cavitation resembling caries (per cent.)
Upper 1st molar . .	30	10·0	0
Upper 2nd molar . .	30	50·0	0
Upper 3rd molar . .	30	3·3	3·3

It is clear, then, that in only one tooth of one animal was there evidence of a lesion simulating coronal caries in man. It may be argued that a higher " caries " incidence would have been found if ground instead of decalcified sections had been made, but it is reasonable to suggest that in most instances any caries would have penetrated the enamel to reach the dentine by the end of the relatively long experimental periods employed. The diets given here are sufficiently similar to some of those arousing a high incidence of caries according to the American workers, but studies of a further series fed on sugar-predominating rations will be reported at a later date.

Finally a similar and fairly common process of cavitation is shown in the upper carnassial tooth root of a ferret (fig. 16), due to parodontal disease of calculous origin. Secondary dentine is seen in the pulp opposite to the calculus-filled peripheral cavities. In the deeper part of the later more penetrating root cavity, calculus is preceded by inflammatory products and in this region gross proliferation of cementum-like material into the pulp has followed secondary dentine as a defensive barrier. It is noteworthy that dental caries is as yet unknown in ferrets.

DISCUSSION

The first parodontal observations on hamsters were recorded incidentally by Keyes (1946a) during studies on dental caries. He drew attention to the accumulation of food and other debris, particularly along the labial surfaces of upper molars and lingual surfaces of lower teeth, together with the impaction of fibrous material interdentally and in occlusal pits and fissures. In addition he noted a supragingival yellow-brown stain inseparable from the enamel cuticle and floating off with it when the latter was released by dilute acid *in vitro*. The condition was ascribed to pigmentation of the cuticle but, in the present writer's opinion, may have been associated with the early phases of calculus deposition. As seen in one of his illustrations (Keyes, 1946a, fig. 18), the presence of calculus and its related parodontal disturbances was clearly encountered. Preliminary observations on parodontal disease in hamsters have been described in rather more detail by Mitchell (1948), and his illustrations closely

resemble some of those shown here and in our previous paper (King and Gimson, 1948-49).   In addition to interproximal impaction of cellulose material, hair and other debris, Mitchell remarked on the frequent occurrence of calculus in the gingival crevices and pockets, with underlying inflammation and loss of alveolar bone.   He did not refer to the sequence of diseased areas in the two jaws and, in contrast to that described here, one of his illustrations indicates a spread of mandibular disease from the third molar forwards.   No mention was made of version of the teeth, nor of lateral bone rarefaction and reciprocal bone deposition, although both conditions can be identified in another maxillary specimen shown.   The experimental period (7 or more months), before which no parodontal lesions were encountered, is also greater than in the writer's experience, but Mitchell gives no clue to the composition of the " laboratory chow " diet he employed. One curious comment is made in his paper (p. 335), namely the suggestion that an apparent increase in disease incidence among those of his animals raised on screen bottom cages might have been due to " the absence of wood shavings, which the animals chew, and which may serve as a gingival stimulator or dental and peridental cleanser, as suggested by King and Gimson (1947) with regard to periodontal lesion production in the ferret."   In our experience, as shown in figs. 8, 9, 11 and 14, the use of sawdust or wood shavings may contribute to rather than prevent the calculus-induced lesions in hamsters, their mastication of these materials being a very different process from the dental and gingival friction supplied by bone-gnawing in ferrets or cane-gnawing in man (King and Glover, 1945 ; King, 1947*a* and *b*).   The prevention of calculus and parodontal disease has not yet been achieved in hamsters.   It may be that some form of frictional prophylaxis will be possible, as in the ferret, but much greater significance is attached to discovering the mechanism of calculus formation itself and its prevention and eradication by other than purely mechanical means.   Some light is shed on the site of origin of this form of parodontal disease due to calculus.   In man, James and Counsell (1927) considered the subgingival epithelium to be the first parodontal tissue to exhibit calculus-induced lesions, but in the carnassial regions of the ferret King (1944) found the gingival crest and gingival epithelium to be primarily affected.   It now seems clear that the site of early parodontal involvement by calculus depends not only on the proximity of the salivary duct orifices but also on the form, occlusion and function of the teeth, the secodont ferret carnassial and human lower incisor regions being associated with primary lesions of the gingival crest and gingival epithelium, while in the bunodont hamster molar and human upper molar areas the crest and subgingival epithelium are first attacked.

Cavitation of the tooth roots during the later stages of parodontal disease in hamsters and ferrets would appear to be largely due to destruction of cementum and dentine by the products of the inflamma-

FIGS. 5 and 6.—Higher magnification of alveolar areas shown in figs. 3 and 4 respectively. Deposition of new palatal bone associated with osteoblast activity (Ob) is seen in fig. 5, while resorption of the alveolus due to osteoclasis (Oc) and to pressure from grossly dilated periodontal vessels (B.V.) is present labially (fig. 6). Some attempt at bone deposition is also seen on the outer part of the labial alveolar crest. × 390.

FIG. 7.—Mesio-distal section through upper M_3 roots of hamster no. 36 ; wholemeal flour basal diet+vitamin-free oil ; experimental period 23 weeks. Note impaction of food particles (FP) and resulting foreign body inflammatory reaction similar to that associated with calculus (Cl), destruction of bone by osteoclasis and pressure resorption due to vaso-dilatation. × 100.

Ehrlich's hæmatoxylin and eosin stain.

PLATE XCIX

PARODONTAL DISEASE IN HAMSTERS
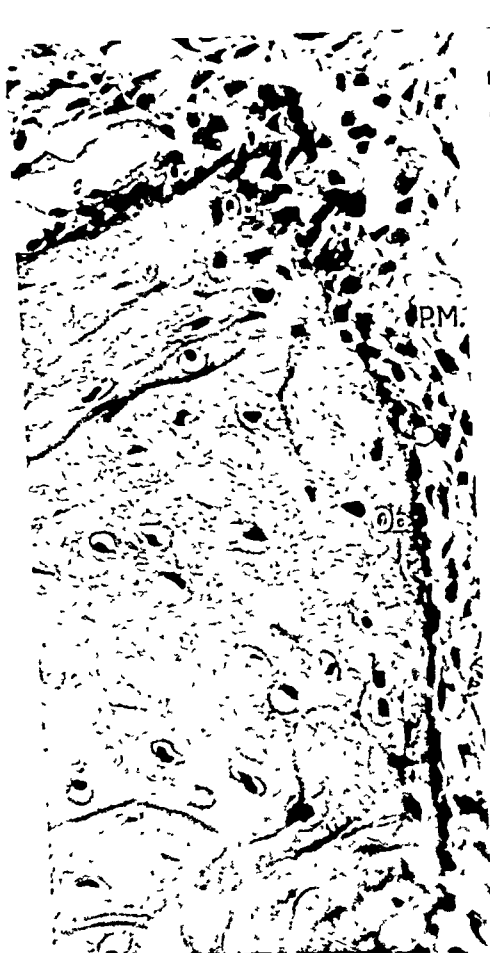
FIG. 5.

FIG. 6.

FIG. 7.

PLATE C

FIG. 8.—Mesio-distal section through upper M_2 and M_3 regions of hamster no. 27; wheatmeal bread basal diet+vitamin D oil; experimental period 26 weeks. Note essential association between enamel cuticle (E.C.) and calculus (Cl.), with resulting penetration and laceration of the gum causing pocket formation and encouraging impaction of food particles (F.P.) and other foreign bodies (W.). × 120.

FIG. 9.—Mesio-distal section through upper M_2 and M_3 regions of hamster no. 26; same diet; experimental period as for animal no. 27 (fig. 8). Here the obliquity of the third molar (M_3) due to its labioversion is shown, together with exposure of much of the M_2 distal root. Excavations (Cv.) of the roots of both teeth, followed by penetration of calculus (Cl.), are seen, together with interdental impaction of cabbage (Cb.) and sawdust (W.) particles. × 60.

FIG. 10.—Mesio-distal section through posterior aspect of upper M_3 of hamster no. 44; wholemeal flour basal diet+cod-liver oil; experimental period 24 weeks. Note broad zone of keratinised subgingival epithelium (K.) due to exposure of latter to the mouth, in which further deposition of calculus (Cl.) is taking place. × 120.

FIG. 11.—Mesio-distal section through upper M_2 and M_3 roots of hamster no. 21; wheatmeal bread basal diet+vitamin D oil; experimental period 64 weeks. Note calculus adherent to obliquely inclined tooth roots and impaction of bread particles (B.P.) and sawdust (W.). Calcification of the bread particles can be seen in some areas. × 60.

Ehrlich's hæmatoxylin and eosin stain.

PLATE C

PARODONTAL DISEASE IN HAMSTERS

FIG. 8.

FIG. 9.

FIG. 10

FIG. 11.

PLATE CI

J. PATH. BACT.—Vol. LXI
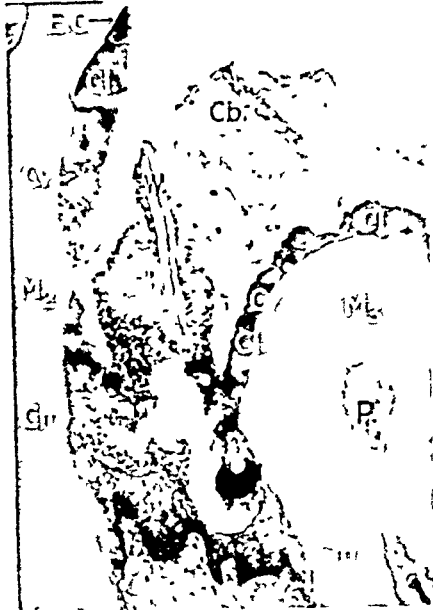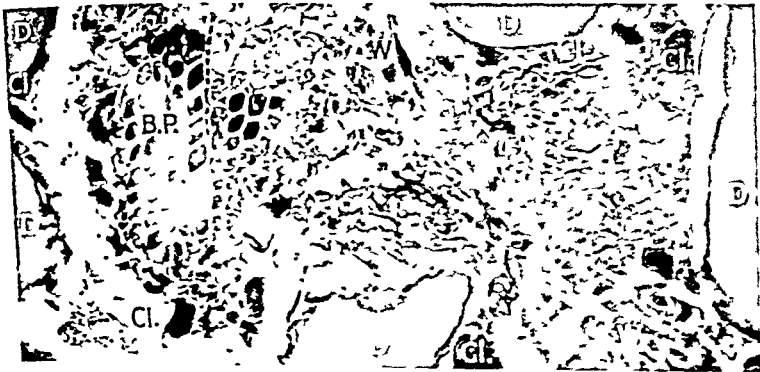
PARODONTAL DISEASE IN HAMSTERS
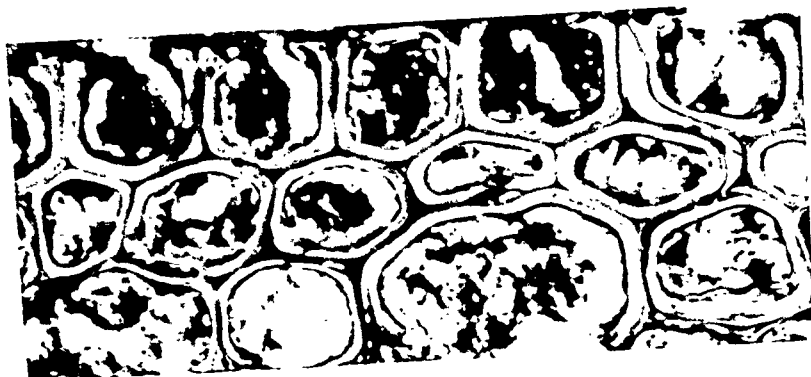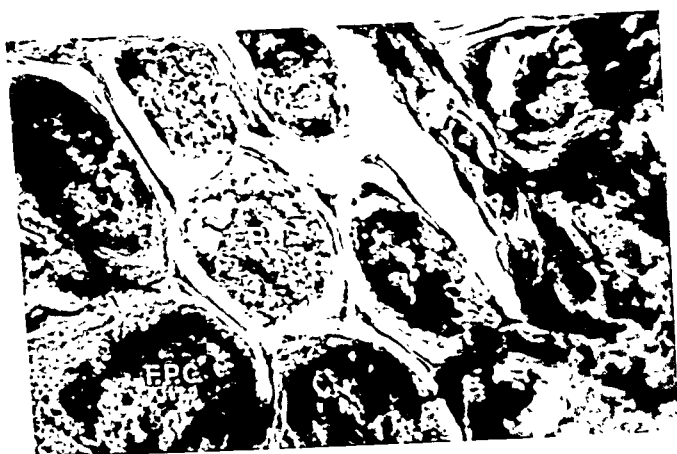
FIG. 12.

FIG. 13.

FIG. 14.

FIG. 15.

PLATE CI

FIG. 12.—Part of mesio-distal section through similar regions of hamster no. 37; wholemeal flour basal diet+vitamin D oil; experimental period 29 weeks. The flour particles are here seen at a stage prior to pathological calcification. × 490.

FIG. 13.—Similar region in hamster no. 38; same diet and experimental period. Examples of pathologically calcified and normal wholemeal flour particles are seen at F.P.C. and F.P.N. respectively. × 490.

FIG. 14.—Mesio-distal section through upper M_2 and M_3 regions of hamster no. 12; wheatmeal bread basal diet+vitamin D oil; experimental period 29 weeks. Note (1) progress of calculus along intact enamel cuticle, (2) cavitation of tooth below amelo-cemental junction, with subsequent penetration of cemental and dentinal cavities by further calculus; and (3) impaction of cabbage particles, some of which are becoming calcified. × 60.

FIG. 15.—Mesio-distal section of distal aspect of upper M_2 of hamster no. 28; wheatmeal bread basal diet+vitamin D oil; experimental period 26 weeks. Note superficial resemblance to dental caries, but lesions here due to excavation of root cementum and dentine by the inflammatory products consequent on deep subgingival proliferation of salivary calculus. × 130.

Ehrlich's hæmatoxylin and eosin stain.

PARODONTAL DISEASE IN HAMSTERS

FIG. 12.

FIG. 13.

FIG. 14.

FIG. 15.

PLATE CII

J. PATH. BACT.—VOL. LXI
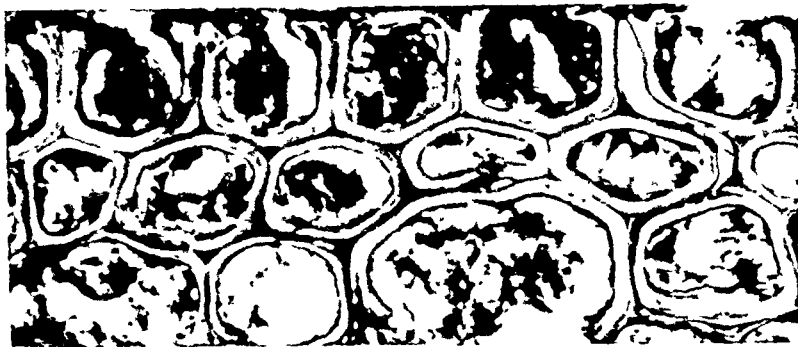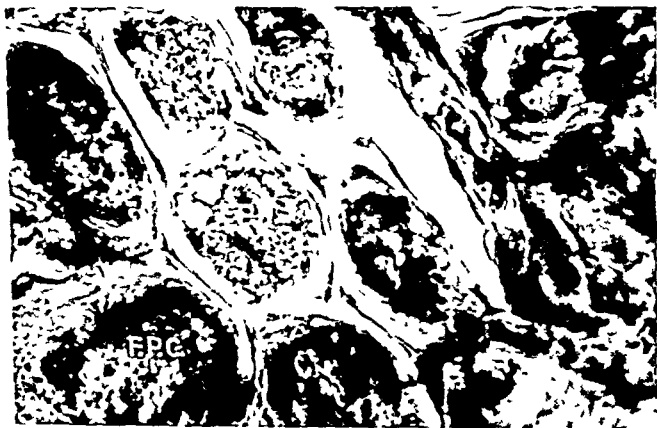
PARODONTAL DISEASE IN A FERRET

FIG. 16.—Labio-palatal section through anterior roots of upper carnassial tooth of a ferret ; nutritionally adequate but bone-free stock bread diet (King and Glover, 1945); experimental period 26 weeks. Note similarity of root excavations to those found in hamsters (figs. 14 and 15). Defensive barriers to the lesions here include both secondary dentine and invasion of the pulp by cementum-like material (Cml.). Weigert's iron hæmatoxylin and Van Gieson stain. ×40.

tion set up in the periodontal membrane just ahead of the proliferating calculus, aided, in the case of the hamster at least, by gingival impaction of food and other debris. The process may be one of proteolysis since the same inflammation in the gum adjacent to the enamel is innocuous to this much more highly inorganic tissue, and the bacterial or enzymic agencies responsible may thus differ from those in caries of human enamel (*e.g.* Pincus, 1937, 1939, 1944). Moreover, root cavitation when present does not affect the whole length of cementum exposed by calculus, a governing factor perhaps being the degree to which the parodontal pockets are pathologically deepened or defensively obliterated. It is also of interest that deposition of calcific material in the form of calculus may border so closely on zones of inflammation and hard tissue destruction. Current histochemical studies suggest that in parodontal disease due to calculus the pathological dental sequelæ beyond the amelo-cementum junction may depend upon the predominance of alkaline phosphatase activity on the one hand and of proteolysis on the other. Excavation of cementum or dentine, or both, point to the temporary local ascendancy of proteolytic factors, while penetration of the resulting cavities by more calculus indicates that the proteolysed debris then forms a suitable pabulum for further phosphatase activity and calculus deposition. Enlargement and deepening of the cavities takes a similar course, penetration by more calculus following closely in the wake of lysis of the tooth root. In the cemental and dentinal cavities, therefore, as in the enamel cuticle (King, 1945) and the pathologically exposed subgingival epithelium and periodontal membrane, elaboration of calculus is related to tissue degeneration. Finally it is of note that some of the writer's specimens of human teeth have disclosed root cavities with calculus penetration similar to those in hamsters and ferrets. Such material may account for some of the cases of rapid " recurrence " of deep calculus in man following careful dental scaling, since the deposits remaining in the inaccessible root cavities would provide excellent foci for further accretions and continued parodontal inflammation.

SUMMARY

1. A description is given of the pathology of a form of parodontal disease in laboratory hamsters maintained on nutritionally adequate rations. The basal diets comprised cereal (wholemeal flour, wheatmeal bread or white maize), separated milk powder, food yeast, sodium chloride, meat and cabbage; experimental supplements included vitamin A- and D-free peanut oil, β-carotene, synthetic vitamin D and cod-liver oil.

2. All of the thirty experimental animals succumbed to parodontal disease of fairly severe degree, irrespective of the type of cereal consumed or of the vitamin A and D intake. It was associated with injury of the gingivæ by salivary calculus, food particles and other debris.

3. The calculous accretions are first observed on the enamel cuticle in the intercuspal grooves of the molar tooth surfaces nearest the orifices of the salivary ducts, and soon progress along the cuticle towards the neighbouring gingival margin to initiate lesions of the parodontal tissues proper. Both calculus formation and parodontal disease therefore arise in the same areas of the denture—labially to the third molar in the maxilla and lingually to the first molar in the mandible.

4. Following penetration of the gingival sulcus by calculus, the course of the disease includes progressive exposure of the subgingival epithelium with keratinisation of its dental aspect, providing a new expanse of enamel cuticle on which further calculus can be laid down; proliferation of the cellular elements of the subgingival epithelium into the corium with associated infiltration of the latter by leucocytes; and the formation of ever-deepening parodontal pockets facilitating gingival impaction of food debris and other foreign bodies.

5. Next, with the progression of calculous and parodontal disease beyond the amelo-cemental junction, inflammation of the periodontal membrane and rarefaction of the adjacent alveolar bone occur around that aspect of the tooth where the primary calculous lesions began. At this stage secondary spread of the disease to other aspects of the initially affected region has begun, together with extension of the process to the approximating structures around the neighbouring teeth. At or about the same time labioversion of the maxillary third molars and, somewhat later, linguoversion of the mandibular first molars begin to result from resorption of their respective external and internal alveolar plates. Such conditions, themselves abetted by the inherent normal inclination of the teeth in the same directions and by the occlusal stresses resulting from the reverse initiation and extension of the lesions in the two jaws, add further impetus to the disease, the final stages of which include cavitation of the roots, sometimes simulating dental caries, and exfoliation of the teeth.

6. There is some evidence that food debris and other foreign bodies may contribute to the disease, not only by direct gingival impaction in the wake of salivary calculus, but also by themselves forming foci for further pathological calcification.

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LOCAL BLOOD-FLOW CHANGES IN EXPERIMENTAL BURNS

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(PLATES CIII AND CIV)

WHEN heat is applied to the skin, two kinds of local change take place, namely direct tissue damage due to heat, and an inflammatory reaction shown by redness, swelling and pain. The influence of this reaction upon the course of the burn is usually ignored and it is commonly held that the sloughing which follows certain burns is due entirely to direct heat necrosis of the skin.

This study is concerned with the changes in the blood flow occurring in burned guinea-pig skin and with the relationship of these changes to skin sensation, œdema and the subsequent clinical course of the burn. Evidence is adduced to show that when stasis of local blood flow occurs, it is associated with analgesia, is related to œdema and causes whole-thickness sloughing of the skin.

The use of dyes in the study of burns

The selective passage of certain dyes from the blood stream into burned areas was recorded by Okuneff (1924). This was confirmed by Kusnetzowsky (1924-25), who used trypan blue, and by Gibson and Brown (1944), who injected Kiton fast green intravenously in four burned patients.

In the present work, burning at known temperatures for known periods was combined with the introduction of the dyes brilliant vital red (colour index 456) or Evans blue (T1824) or both into the general circulation before or after burning or both.

These dyes were selected because they are non-toxic, are of contrasting colours, combine firmly with the albumin of the plasma (Gregersen *et al.*, 1935 ; Gregersen, 1949, personal communication), and remain within the circulation for hours. If dye-stained plasma is in the blood stream when a local increase of capillary permeability occurs, the dye passes out of the affected vessels and stains the local tissues. If the blood flow through the permeable vessels slows down and subsequently ceases and dye is then introduced into the general

circulation, staining of the affected area does not occur. Such local reactions of the burns to the intracardiac injection of these dyes either before burning or at known intervals after burning were related to the temperature and duration of burning, the subsequent clinical course of the burn and other factors.

EXPERIMENTAL METHODS

Constant temperature burning iron (figs. 1 and 2). This was a hollow brass cylinder two in. high, one in. in diameter and closed at one end. A continuous supply of hot water circulated through the iron via inlet and outlet tubes, the former passing almost to the bottom of the iron (fig. 1). The temperature of the water near the bottom of the iron was read from a thermometer inserted through a rubber stopper in the top of the cylinder. The circular bottom of the iron, which was the burning surface, was smooth and polished; the other surfaces were lagged with rubber. It was assumed that the temperature of the burning surface was that recorded on the thermometer. Any temperature between 40° and 80° C. could be maintained for minutes within 0·5° C. by altering both the temperature of the water supply and the flow through the iron. With this iron, circular burns one inch in diameter and caused by a known temperature were obtained. All temperatures are recorded in degrees centigrade.

Burning and animals. The burns were made under open-ether anæsthesia by firmly pressing the iron to the guinea-pig's back or abdomen. The duration of burning ranged from 10 secs. to 20 mins. but usually was either 10, 20, 30 or 60 secs., timing being carried out with a stop-watch. Burning temperatures ranged between 43° and 80°, but over the range of 52° to 76° burns of 10, 20, 30 and 60 secs. duration were inflicted at intervals of 1°. Most of the burns were repeated at least once and the results were consistent.

In all, 320 burns were inflicted on 68 albino guinea-pigs of 18-40 ounces wt. The number of burns per animal ranged from 2 to 10 but was usually 4 or 5. The larger numbers (6 to 10) were inflicted only when the animal was to be killed the same day. One or two days before burning, the back, flanks and abdomen of the animal were clipped, and either shaved or depilated with barium sulphide cream.

Skin temperature determination. Repeated skin temperature records of the edges and centres of 16 burns (in 4 animals) were made with a resistance type electrical recording skin thermometer (Light Laboratories, Brighton).

Dyes. Evans blue (T1824, I.C.I.) and brilliant vital red (colour index 456, National Aniline Co., New York), were made up in 1 per cent. aqueous solutions and autoclaved. They were injected intracardially in doses of about 1 c.c. In many experiments only Evans blue was injected, either 10-20 minutes before burning or ½-5 hours after burning. In other experiments, the red dye was injected either before or after burning and the blue dye some hours later.

Miscellaneous. Very few animals died from the burns but a few died from cardiac puncture. The burns were left open to the air and none became septic.

EXPERIMENTAL OBSERVATIONS

Reactions at the site of burning

The following descriptions apply to the use of both dyes, but for the sake of brevity the blue dye is referred to in the text unless the ---1 dye is specifically mentioned.

PLATE CIII

FIGS. 1 and 2.—Constant temperature burning iron.

FIGS. 3 and 4.—Correlation of œdema, stasis of the blood flow and
sensory loss in burns.

The guinea-pig's abdomen was pencilled into quadrants and burned at 62° C. for
$\frac{10 \mid 20}{30 \mid 60}$ seconds. In fig. 3, photographed ¾-hour after burning, marked œdema
was seen to have developed in the lower burns but was slight in the upper. Tests
showed normal skin sensation in the upper burns and analgesia in the lower.

Evans Blue was injected intracardially 1 hour after burning. In fig. 4, photographed
20 minutes later, the upper burns have developed patch reactions, indicating
continuation of the local blood flow; in the lower, ring-with-erythema reactions
indicate that stasis had occurred.

CONSTANT TEMPERATURE BURNING IRON

SHOWN (1) IN SECTION
AND   (2) FROM BELOW, = BURNING SURFACE

OUTLET   INLET

(1)   R   R

T = THERMOMETER
R = RUBBER INSULATION

(2)   R   R
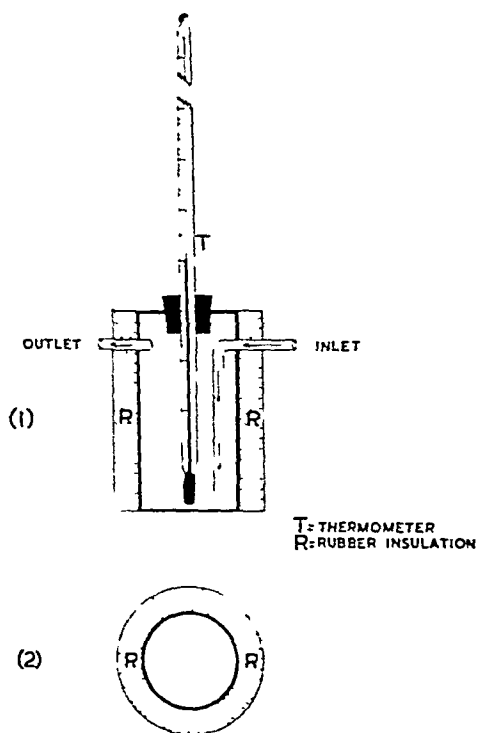
FIG. 1.
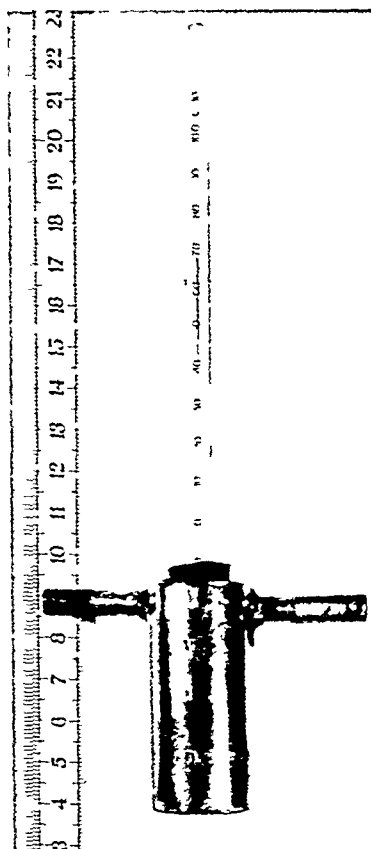
FIG. 2.

FIG 3

FIG. 4

Four types of reaction were noted. When the injection of dye preceded burning the following occurred in the burned area.

Erythema. The occurrence of local erythema without diffusion of the dye was characteristic of burns of minimal severity.

Patch reaction. In other burns, erythema was succeeded by a faint blue colouration of the skin which deepened in colour and developed into a localised circular area of deep blue skin corresponding with the site of application of the burning iron. This is referred to as the *patch reaction* (*e.g.* top left burn in fig. 6).

Ring reaction. A different reaction occurred in more severe burns, dye appearing at the periphery of the burn in the form of a ring. Typically the site of application of the heating iron was unstained, often it was erythematous, but at higher temperatures it was stiff and yellow, due to heat necrosis of the skin. This is called the *ring reaction.*

Ring-with-erythema reaction. When dye was injected after burning, the patch or ring reaction often occurred. In other burns, however, another type of reaction took place. This resembled the ring reaction in that a coloured ring appeared at the periphery of the burn within a few minutes of injecting the dye, but the burn itself was always erythematous. The central reddening contrasted with the peripheral blue outline and for this reason it is referred to as the ring-with-erythema reaction (*e.g.* lower burns in figs. 4 and 6).

Ring-with-erythema reactions : stasis and stagnation

This reaction occurred, for example, in a burn at 61° for 1 minute when the dye was injected 4 hours after burning, but a patch reaction occurred in an identical burn on another animal when the dye was introduced before burning. The blue patch with Evans blue before burning in one animal indicated that, soon after burning, the blood was flowing through capillaries of altered permeability : but the failure of dye to appear in the erythematous area 4 hours after burning in the other animal indicated that the flow had ceased under the site of application of the burning iron, although it was continuing, with increased capillary permeability, in a narrow zone at the periphery. Pressure on the skin with a glass slide failed to obliterate the central erythema and the skin temperature at the centre of the burn was often 0·5° to 1° less than that of the edge. Cessation or stasis of the blood flow was therefore confirmed.

The development of stasis in a single burn was shown by the use of the two dyes introduced at different times in one animal. For example, brilliant vital red was injected intracardially and burns at 61° for 10, 20, 30 and 60 seconds were then inflicted on the guinea-pig. Each burn gave rise to a red patch reaction. Three hours later Evans blue was injected intracardially. In the 10- and 20-seconds burns the red patch reactions were replaced by blue, indicating that blood

was still flowing through dermal vessels of altered permeability. In the 30 and 60 seconds burns, however, a blue ring developed at the periphery of each red patch reaction, which were otherwise unchanged. It was concluded that while blood was continuing to flow in the zone of graded vascular damage at the periphery of the burns, stasis had occurred in the area underlying the site of application of the burning iron.

The temporary ring-with-erythema reaction : stagnation

Some of the ring-with-erythema reactions remained unchanged for hours. In others, changes occurred within an hour of injection of the dye, often within 10 or 20 minutes. In these, the dye gradually spread inwards from the blue edge, encroaching upon and finally often obscuring the central erythema. For example, Evans blue was injected 1 hour after burning at 65° for 20 seconds. Five minutes later a typical ring-with-erythema reaction had developed, at 20 minutes the dye was spreading inwards from the edge, and within an hour the central erythema was hidden. This type is called the *temporary ring-with-erythema reaction*.

The nature of this reaction suggested that stasis had not occurred but that the blood flow through the burn had become greatly retarded, in other words that stagnation was present. This was confirmed by the observation that, in burns of the same duration in one animal, the temporary reaction occurred at burning temperatures just below those at which stasis developed and above those at which patch reactions occurred.

Time of onset of vascular stasis

To determine how rapidly stasis of the dermal blood flow develops and whether the time of onset is related to the temperature and duration of burning, similar burns were inflicted on a number of animals, one of which had previously been injected with Evans blue. The dye was introduced into the others at different intervals after burning, usually $\frac{1}{2}$, 1, 2 and 4 hours. In some burns patch reactions occurred, in others temporary ring-with-erythema reactions and in yet others " permanent " ring-with-erythema reactions. By comparing the reactions of similar burns in these animals, the time limits within which stasis developed were determined, and these were compared for burns of increasing severity.

For example, in 30-second burns at 56° patch reactions occurred whether the dye was introduced before burning or 1-4 hours after burning, indicating the continued circulation of the local blood flow for at least four hours after burning. At 58° to 60°, however, patch reactions were obtained when the dye was introduced both before and up to 2 hours after burning, but when the delay before injection was 4 hours, temporary or permanent ring-with-erythema reactions

were obtained. Stagnation or stasis of the blood flow therefore was not present within the first two hours after burning but developed within the following two hours. At 62° and 64°, patch reactions occurred when the dye was introduced before burning, but ring-with-erythema reactions occurred with its introduction half-an-hour after burning or later. Stasis, therefore, must have set in within half-an-hour of burning. At 67° and 68°, reactions identical with ring-with-erythema reactions occurred when the dye was injected before burning, and stagnation or stasis must have set in during the period of application of the burning iron, *i.e.* within 30 seconds. Comparable results were obtained with burns of 10, 20 and 60 seconds.
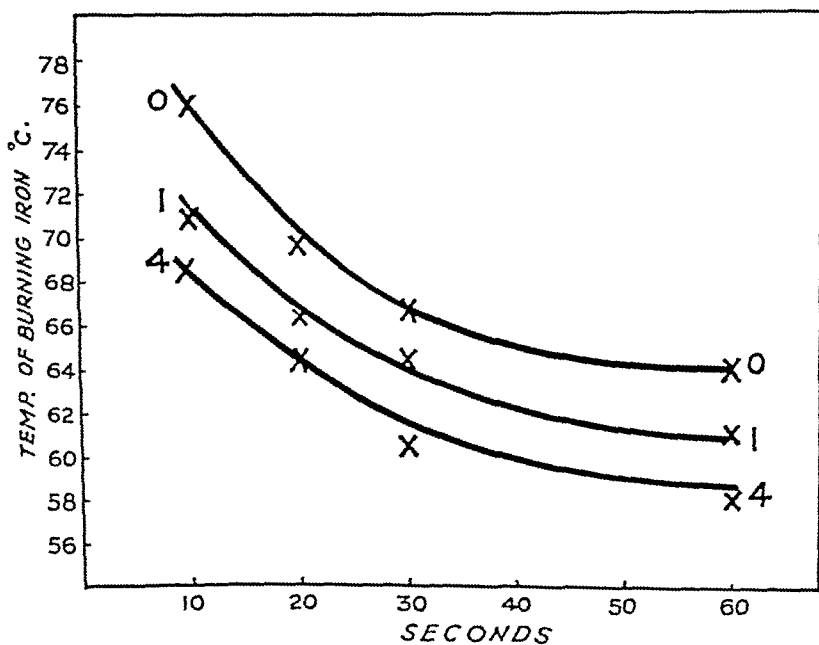
FIG. 5.—The curves show the minimal temperatures and times of burning which produce :—
    (*a*) stagnation during the period of burning (0—0).
    (*b*) stasis within 1 hour of burning (1—1).
    (*c*) stasis during the following 3 hours (4—4).

In figs. 5 and 10 (p. 438), the curves are based on the results of many burns of 10, 20, 30 and 60 seconds duration at temperatures below, at and above the temperatures recorded. The points upon which the curves are based are the mean points of narrow temperature zones of about 2°, so that the error of each curve is about ±1°.

Stasis, therefore, may set in hours, minutes or seconds after burning, the interval decreasing as the severity of burning increases, until it develops within the burning period. This relationship is shown in fig. 5, where the three curves give the minimal temperatures and times of burning which produce (*a*) stagnation during the period of burning, (*b*) stasis within 1 hour of burning and (*c*) stasis within 4 hours of burning. From these curves the various temperatures of burning in burns of constant duration which are required to produce

these changes in the blood flow can be ascertained. Similarly the various periods of burning required in burns of constant temperature can be read off. For example, in burns of 15 seconds, stagnation sets in during the burning period when the burning temperature is 73°, within 1 hour of burning when the burning iron is between 69° and 73° and within the next three hours when the temperature is 66° to 69°.

Relationship of stagnation and stasis to burn œdema

The passage of Evans blue or brilliant vital red into a burn is associated with the exudation of a variable quantity of fluid which, above a certain level, gives rise to clinical œdema. It has been shown that stasis in a burn is preceded by a variable period of time during which blood continues to flow through capillaries of abnormal permeability. During this period the blood flow becomes progressively retarded until stagnation is manifest.

Cyanosis. Retardation of the blood flow, however, may occur without the development of stagnation or stasis and may be detected by the development of cyanosis in the burn erythema. Cyanosis of peripheral origin is due to greater reduction of oxyhæmoglobin by the tissues (Lundsgaard and Van Slyke, 1923) and is due, in peripheral cyanotic erythema, to slowing of the blood flow. Cyanosis was observed in many burns during the period preceding the injection of the dye and was absent in others. It occurred in all burns giving rise to a ring-with-erythema reaction as well as in some burns failing to give this reaction.

Œdema and blood flow (figs. 3 and 4). The relationship of burn œdema to retardation of the blood flow was studied by recording the degree of clinical œdema at various times after burning in a series of burns at known temperature and of known duration. The observations were related to the occurrence of cyanosis and to the rapidity of onset of stagnation and stasis as determined by the dye technique. The results recorded in table I were obtained from 29 burns of 1 minute's duration, and comparable results were found in burns of 10, 20 and 30 seconds.

In the 53° and 55° burns œdema did not occur, and the blood was still flowing through the dermis 4 hours after burning, even when slowing of the blood stream was detected (cyanosis present in 55° burn). At 56°, œdema developed slowly but was never marked, and stagnation was detected 2 and 4 hours after burning. At 58°, œdema developed within an hour and increased rapidly during the next hour, while stasis did not occur during the $1\frac{1}{3}$ hours following burning but took place within the next $2\frac{1}{2}$ hours. At 59°, 60°, 61° and 63°, well-marked œdema developed with increasing rapidity, as did the onset of stagnation and stasis.

It is clear, therefore, that clinical œdema occurs only in burns in which stagnation develops, and that the rate and degree of accumula-

tion of œdema fluid is related to the rapidity of onset of this change in the blood flow. Œdema is absent or minimal when stagnation

TABLE I

Relationship of burn œdema to the development of stagnation and stasis : burning iron applied for 1 minute

Temp of the burning iron (° C)	Condition of the local blood flow as determined by the reaction of the burn to the intracardiac injection of Evans blue at various intervals after burning	Burn cyanosis	Clinical estimate of the burn œdema at various intervals after burning
53°	" Active " circulation at 2 and 4 hrs. after burning	—	Œdema absent
55°	" "	+	" "
56°	Stagnation at 2 and 4 hrs. after burning	+	— at ½ an hr., + at 1½ hrs., +- +- at 2 hrs. after burning
58°	" Active " circulation at 1½ hrs., stasis at 4 hrs.	+	± at ½ an hr., +- at 1 hr., +++ at 2 hrs.
59 and 60°	Stagnation at 1 hr., stasis at 4 hrs	+	++- at ½ an hr., +++ at 1, 2 and 4 hrs.
61°	Stasis at 1 hr.	+-	+-+- at ½ an hr.
63°	Stasis at ½ an hr.	+-	+++ in 10 mins.

" Active " local circulation = the patch reaction : stagnation and stasis = appropriate ring-with-erythema reaction : œdema marked, +++-; œdema visible, ++-; œdema palpable, +; œdema slight ±.

cannot be detected : when œdema develops rapidly and abundantly the blood flow is stagnant and usually ends in stasis (figs. 3 and 4). Slowing of the blood flow not amounting to stagnation is not associated with œdema.

Stasis of the blood flow in burns and its relationship to analgesia and subsequent whole skin loss

Stasis and analgesia

When the animals had recovered from the anæsthesia, the sensibility of the burned skin was determined by comparing the reactions of the animal to squeezing the burned and local unburned skin with forceps. When sensation was normal, the guinea-pig " jumped," when analgesia was present the animal ignored the stimulus. Partial loss of sensation was inferred from the animal's lessened reaction. Tests were made 2-4 times during the six hours following burning.

In table II is set out the relationship between sensation in 111 burns and the state of their local blood flow ½-5 hours after burning as determined by the dye technique. Only burns in which the local circulation continued for some time after burning are included, *i.e.* only burns which would have given a patch reaction had the dye been injected before burning.

All 44 burns giving permanent ring-with-erythema reactions remained insensitive on repeated testing, *i.e.* stasis of the blood flow

was always associated with analgesia. Of 22 burns giving the temporary ring-with-erythema reaction of stagnation, 10 were analgesic within $\frac{1}{2}$-1 hour of burning and 3 were normally sensitive during the period of test : in 9, however, the results of testing showed a change

TABLE II

Local circulation in burns and burn sensibility

Objective pain sensation	Reactions of the burns to the injection of dye $\frac{1}{2}$-5 hrs. after burning		
	Patch	Temporary ring-with-erythema	Ring-with-erythema
Normal	39	3	0
Changing * . . .	5	9	0
Absent	1	10	44
Totals . . .	45	22	44

* See text

Numerals refer to numbers of burns

with time, that is to say, after a period of normal or impaired sensation, analgesia or diminished sensibility developed. In 39 of 45 patch-reacting burns sensation remained normal ; in 1, analgesia was early and permanent ; in 5, sensation changed with time, partial or normal sensibility progressing to analgesia in 4 and recovering after temporary impairment in 1.

It has been shown that stasis may take hours to develop, and that the rapidity of its onset is related to the severity of the burn. This is probably the explanation of the burns in which patch or temporary ring-with-erythema reactions occurred and in which analgesia was demonstrated, as they were severe enough to have shown the reaction of stasis had the dye been injected 2 or 3 hours later.

Three of the burns in which stagnation was detected retained a permanently normal sensation. As whole skin loss did not subsequently develop (*vide infra*) it is almost certain that stagnation of the dermal blood flow in these cases was temporary and later returned to normal.

It can be said, therefore, that the development of permanent analgesia in a burn indicates that stasis of the blood flow has or will set in, and that the continued retention of normal sensation indicates that active circulation of the local blood flow will continue. It would appear, however, that the onset of analgesia and stasis may not be simultaneous, analgesia often preceding stasis.

Skin circulation and depth of skin loss

Many burns were examined every 2-5 days for a month or longer. Four categories of burns, distinguished by thickness of slough, mode

of re-epithelialisation and time of healing were recognised, namely: no skin loss, superficial skin loss, partial skin loss and whole skin loss.

Superficial skin loss (s.s.l.). A thin rubbery slough formed which could be rubbed off in 7-10 days, revealing a completely healed burn.

Partial skin loss (p.s.l.), (*e.g.* the top left burn in figs. 6-8). The slough varied in thickness and when it was removed a red shallow ulcer was exposed containing islands of epithelium from which rapid healing occurred.

Whole skin loss (w.s.l.) (*e.g.* the lower burns in figs. 6-8). A thick slough formed and when it was removed after 10-14 days, a bleeding and often deep ulcer was exposed. Epithelialisation took place only from the edge of the ulcer.

Stasis and whole skin loss

Seventy-two burns in which the state of the local blood flow had been determined by the dye technique $\frac{1}{2}$-5 hours after burning were classified according to the depth of skin loss. The relationship is set out in table III. More severe burns which would have given ring

TABLE III

Local circulation in burns and subsequent depth of skin loss

Subsequent depth of skin loss	Reactions of the burns to the injection of Evans blue $\frac{1}{2}$-5 hrs. after burning			Totals
	Patch	Temporary ring-with-erythema	Ring-with-erythema	
Nil	8	0	0	8
Superficial skin loss . .	15	0	0	15
Partial skin loss . .	10	6	0	16
Whole skin loss . .	3	5	25	33
Totals . . .	36	11	25	72

Figures refer to numbers of burns

reactions had the dye been injected before burning are excluded from the analysis, as it was shown that such burns are invariably w.s.l. burns. All the 25 burns producing permanent ring-with-erythema reactions and only 3 of 36 patch-reacting burns went on to w.s.l. The severity of these 3 burns suggested that had the dye been injected later, ring-with-erythema reactions would have occurred. The remainder of the patch-reacting burns suffered either no skin loss, superficial loss or at most partial skin loss.

Of the 11 burns in which stagnation was detected, only 5 developed w.s.l., in the remainder the loss was partial. In these w.s.l. burns, stasis must have followed stagnation of the blood flow, but in the other six, stasis could not have occurred through the full depth of the skin. Recovery of normal blood flow in the deeper part of the

dermis must have taken place, otherwise it would have sloughed, and healing from dermal epithelium would have been impossible.

Thus the depth of skin loss is closely associated with the state of the dermal blood flow. When blood continues to circulate, whole skin loss does not occur, but the onset of stasis is followed by sloughing of the entire depth of the skin.

Sensation and skin loss

A separate analysis of the sensibility in 101 burns and the subsequent depth of skin loss was made.

Of 46 w.s.l. burns, analgesia was early and permanent in 43 ; in the others, the onset was delayed for some hours. On the other hand, normal sensation was retained in all the 31 burns in which skin loss was absent or superficial. Variable results were obtained with the 24 p.s.l. burns. In 10, sensation was normal, in 2 it was absent and in 12 it changed during the first six hours after burning. In these, sensation either returned after early loss, or analgesia developed after some hours.

Thus the result of testing pain sensation in a burn is, in general, a reliable index of its future course. If the stimulus can be felt some hours after burning, the loss will at most be partial and epithelial healing will take place from the epidermis or dermis of the burned skin. The presence or development of analgesia, however, indicates the probable development of whole skin loss.

Example of an experiment to show the relationship between stasis, analgesia and whole skin loss

The results of one experiment which showed the relationship between the blood-flow changes in burns, skin sensibility and sub-sequent depth of skin loss are shown photographically in figs. 6-9. All the photographs are of the same guinea-pig. The abdomen was pencilled into quadrants and circular burns were inflicted, the temperature (° C.) and duration (seconds) of which were as (a). Tests of skin sensation revealed these results, namely (b) where
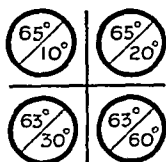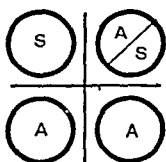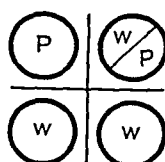
(a)   (b)   (c)

S = sensation normal and A = analgesia. It is to be noted that the 65° for 20 seconds burn was uneven, due to temporary slipping of the burning iron, half of the burn being analgesic and half sensitive.

LOCAL BLOOD FLOW IN EXPERIMENTAL BURNS
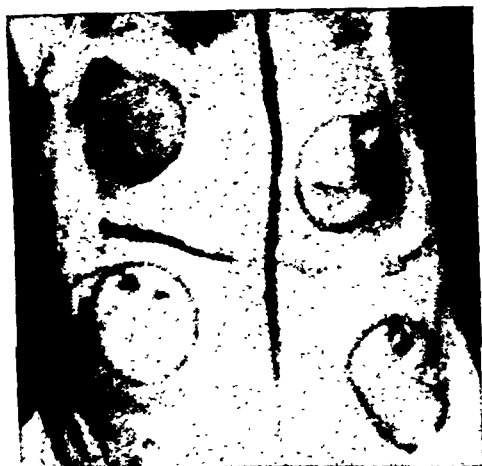
FIG. 6.—Evans blue injected 3 hours after burning : photographed 1 hour later.
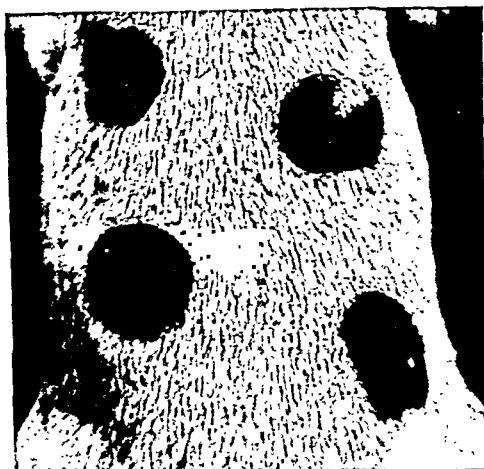
FIG. 7.—Same burns 5 days later.
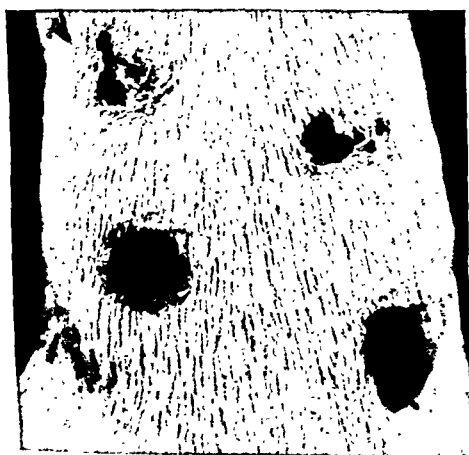
FIG. 8.—12 days after burning.

FIG. 9.—19 days after burning.

FIGS. 6-9.—RELATIONSHIP OF THE LOCAL BLOOD FLOW IN BURNS TO SENSATION AND SUBSEQUENT DEPTH OF SKIN LOSS. (For explanation see text, p. 436.)

Three hours later, Evans blue was injected intracardially. In fig. 6, the 65° for 10 seconds burn has developed a patch reaction indicating continuation of the dermal blood flow, both of the 63° burns developed ring-with-erythema reactions indicating stasis of the dermal flow and half of the 65° for 20 seconds burn showed a patch reaction, the other half a ring-with-erythema reaction.

The subsequent course of the burns is shown in figs. 7-9. Sloughs had formed by the 5th day and were removed on the 12th. By then, almost complete epithelial healing from the floor of the burn had occurred in the 65° for 10 seconds burn. In the lower two burns granulating ulcers had formed, healing was by epithelial ingrowth from the edge and skin cover was slow. Half of the 65° for 20 seconds burn was epithelialised from the floor within 12 days and in the other half healing occurred from the edge of the burn. The results may be represented schematically as in (c) (p. 436), where P and W = partial and whole skin loss.

Relationship of severity of burning to the triad of stasis, analgesia and whole skin loss : dermal threshold temperature

The minimum temperatures for burns of 10, 20, 30 and 60 seconds at which (1) stasis set in within 4 hours of burning, (2) analgesia of the skin occurred and (3) whole skin loss developed are all plotted in fig. 10 and three appropriate curves drawn. These are strikingly similar and, allowing for experimental error, may be considered identical. Thus in burns of between 10 and 60 seconds duration, a series of minimal temperatures exists at which local vascular stasis, analgesia and whole skin loss occur. If this relationship holds good for burns of longer duration, then in a theoretical burn of infinite duration there must be a minimal burning temperature at which sensation is lost, vascular stasis develops and whole skin loss occurs.

Extension of the curves to the right indicates that this minimal temperature lies between 50° and 55°. The actual temperature has not yet been accurately determined but these limits have been confirmed in burns of 2-10 minutes duration.

Mendelssohn and Rossiter (1943-44) have shown that the equilibrium subcutaneous temperature obtained with a prolonged burn at 50° is 45° and with burning at 55° it is 50°. Thus the equilibrium subcutaneous temperature of a burn between the above limits is between 45° and 50°. The mean temperature of the dermis must be somewhat higher than this, say between 48° and 52°. This may be the lowest or threshold temperature to which the dermis must be heated before stasis, analgesia and whole skin loss will follow. If this explanation is correct, the threshold temperature must be reached in 60 seconds when the burning iron is at 58° to 59°, as this is the lowest temperature at which the above triad develops in 1-minute burns (fig. 10). From Mendelssohn and Rossiter's data, the sub-

cutaneous temperature 1 minute after applying the burning iron at 60° was about 48·4°. This supports the theory that unless the dermis
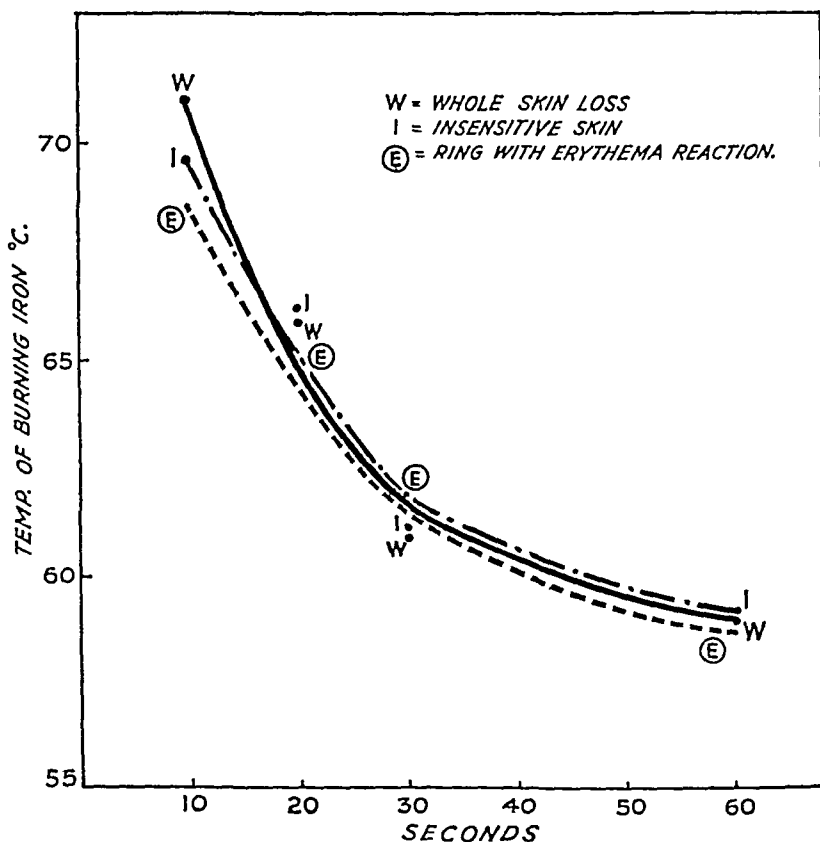
Fig. 10.—The three curves show the minimal temperatures and times of burning which produce stasis within 4 hours of burning, analgesia of the skin and whole skin loss. The curves are strikingly similar and may be considered identical.

is heated to a minimal or threshold temperature during burning, the triad of stasis, analgesia and whole skin loss will not occur.

DISCUSSION

Galen (130 to *circa* 210 A.D.) was the first to record that inflammation could be caused by many agents, including heat, but it was not until the classical work of Cohnheim (1873, 1889) that the underlying vascular changes were investigated. His studies included the changes in the blood flow which followed immersion of the rabbit's ear in water at different temperatures. He showed that, after an initial acceleration, slowing of the local blood flow occurs, that this is associated with the exudation of fluid and may be followed by stagnation and stasis.

In studies of frost-bite, Kreyberg and Rotnes (1931), Greene (1942, 1943), Lange and Boyd (1945) and Kreyberg (1946, 1949) have

emphasised that the hyperæmia and œdema present are part of an acute inflammatory reaction in the affected part : if freezing is severe enough, stagnation and stasis of the blood flow occur. We have confirmed that in the inflammation of burns similar changes may take place, so that the changes produced by heat and cold injuries have much in common.

Stasis and whole skin loss

Let us consider the relationship between vascular stasis and whole skin loss. Kreyberg and Rotnes (1931) and Kreyberg (1946) showed that when stasis of the blood flow occurs after frost-bite, necrosis of the affected part follows, the necrosis being due to permanent tissue anoxia. It may be argued that, although there is a correlation between vascular stasis and skin sloughing in burns, the latter does not depend on cessation of the blood flow. Glenn (1944) suggested that the distension of the tissues due to burn œdema may interfere with the nutrition of the skin and be responsible for extension of the tissue destruction. We have shown, however, that in guinea-pig burns clinical œdema only occurs when there is extreme retardation of the blood flow and this commonly ends in stasis. Glenn's observations on tissue destruction may therefore be related, not to the œdema, but to the associated development of stasis. Further, it may be argued that both stasis of the blood flow and whole thickness sloughing of the skin are due to the direct effects of heat, or indeed that heat necrosis or necrobiosis may precede and cause the stasis. However, we have seen that cyanosis occurs in the burns in which retardation and finally stasis of the blood develops. Cyanosis indicates respiratory activity and therefore some elements of the skin must have been alive for some time after the removal of the burning iron. Furthermore, Leach *et al.* (1943-44), in a histological study of guinea-pig burns, have shown that there is a vertical distribution of skin changes of decreasing severity, and this has been confirmed by us for many guinea-pig and human burns. For example, in a burn at 65° for 1 minute, excised 2 hours after burning, Leach *et al.* have shown that the surface of the skin is rendered necrotic and that the epithelial changes are irreversible superficial to a depth of 0·12 mm. However, at and below a depth of 0·37 mm. from the surface, changes in the hair follicles are slight or absent. Nevertheless, the whole thickness of the skin invariably sloughs in such a burn. Instead of forming foci of epithelial regeneration, the viable follicles finally become necrotic. We believe that this is an ischæmic change due to stasis.

Heat can directly produce irreversible changes in the whole skin, however, if the thermic injury is severe enough, and we agree that whole thickness sloughing of many burns is due to direct heat necrosis. We recognise, therefore, two kinds of whole skin loss burns, namely those due to direct heat necrosis of the dermis, and those due to stasis

of the blood flow, the latter commonly associated with superficial heat necrosis. In the former the skin is avascular, whitish or yellow, and possibly charred : in the latter inflammatory redness and swelling occur.

The blood cells in stasis

The changes in the blood leading to stasis may be observed directly by examining the minute vessels in the burned area with a microscope. The blood is at first seen running quickly through all the minute vessels of the skin but as the flow becomes stagnant and then static, the red cells adhere in clumps and are found as tightly packed masses in the capillaries and veins. This process, however, is not thrombosis. Tannenberg and Fischer-Wasels (1927, quoted by Kreyberg, 1949) state that the clumped red cells can separate and individually re-enter the blood flow. Histological examination of burned skin in which stasis of the blood flow was diagnosed by the dye technique shows the capillaries tightly packed with blood corpuscles. The individual structure of the corpuscles appears unchanged if the skin is excised soon after the onset of stasis, but if this is delayed for several hours, confluent intravascular, eosinophilic masses, usually interpreted as thrombi, are to be found within the capillaries and venules. There is some doubt, however, whether these structures are true thrombi. It is possible they are actually clumps of blood cells undergoing necrosis, this necrosis being part of the general necrotic change occurring in the area affected by the stasis.

Reversibility of the blood flow changes

Brown and Landis (1947), who produced stasis in the frog by freezing, claim to have observed the removal of plugs of packed corpuscles by in-flowing blood, and the resumption of a normal blood flow in the vessels. Although Kreyberg (1949) observed the dissolution of stasis in individual vessels, this only occurred to a slight extent and did not affect the whole part.

Stasis in guinea-pig burns is invariably followed by whole skin loss, which would suggest that, in burns, it is an irreversible change. It is important to establish, however, whether the irreversibility is due to stasis *per se* or to subsequent thrombosis. Lange and Boyd (1945) claim to have prevented the necrosis which normally follows the stasis due to severe frost-bite by heparinising their animals. This was not confirmed by subsequent workers (Quintanilla *et al.*, 1947 ; Schumacker *et al.*, 1947).

Induced reversibility of stasis in frost-bite therefore is still an open question : as far as we are aware it has not been investigated in burns.

With regard to the reversibility of stagnation, the question is less in doubt. In a number of burns in which stagnation of the blood

flow was detected we have seen partial and not whole skin loss sub-
sequently develop. This suggests either that stasis of the blood flow
did not follow stagnation, or if it did that it was localised to the
superficial part of the dermis. Recovery of the normal blood flow
in the deeper part of the dermis must have taken place, otherwise
healing from dermal epithelium would have been impossible. This
indirect evidence of the reversibility of stagnation needs to be con-
firmed by a more direct method, *e.g.* by the detection, with the double
dye technique, of a patch reaction in a burn subsequent to the
occurrence of a temporary ring-with-erythema reaction.

SUMMARY

1. The effects of experimental burns produced in guinea-pigs by
known temperatures applied for known periods were studied and the
consequent qualitative blood flow changes in the skin were investigated
by the use of circulating Evans blue or brilliant vital red or both,
injected either before or after burning. The results were correlated
with the burning temperature and time, with the sensory changes
in the burns, with the development of oedema and cyanosis and with
the subsequent depth of skin loss.

2. Continuation of the dermal blood flow in a burn was accompanied
by normal skin sensation and by the absence of both clinical oedema
and subsequent whole skin loss. The development of stasis was
associated with clinical oedema and analgesia and followed by whole
skin loss.

3. A dermal threshold temperature is postulated at and above
which the triad of stasis, analgesia and whole skin loss develops.

4. Some of the implications of stasis in burns are discussed.

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SHORT ARTICLES

THE STAINING OF ERYTHROCYTES IN TISSUE SECTIONS

A NEW METHOD AND OBSERVATIONS ON SOME OF THE MODIFIED MALLORY CONNECTIVE TISSUE STAINS

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Specific demonstration of the erythrocytes in sections is occasionally desired by practically everyone who labours under the ambiguous title of animal histologist. Probably the peak of selectivity is achieved by the Pickworth methods (Mallory, 1938) which, in frozen sections of brain at least, can provide an unquestionable demonstration, but unfortunately there has been no method of equal specificity applicable to paraffin sections.

For the effective demonstration of erythrocytes in tissue sections from paraffin it is, of course, essential to have reasonably fresh tissue because after death the hæmoglobin escapes from the erythrocytes and it is only the intact erythrocyte which shows a useful affinity for dyes.

The choice of fixative is similarly of fundamental importance because many otherwise excellent fixatives can destroy the integrity of the erythrocyte as effectively as post-mortem change. The alcoholic fixatives are all lytic for blood except in very thin sections of tissue, as are also all the more acid aqueous fixatives, including mercuric chloride and such mixtures as Zenker's and Bouin's (with its modifications) and Heidenhain's Susa solution. For really fresh tissue formol-sublimate (Lendrum, 1947) and Zenker-formol are suitable, but if post-mortem lysis has begun, then a plain formalin mixture (formol-saline, Kaiserling or Pick-Jores) will fix the erythrocytes with the least amount of additional lysis. There is, however, one serious disadvantage of the formalin fixatives for post-mortem material, the formation of so-called formalin deposit (Lillie and Hershberger, 1947), which, it should be remembered, may be formed in fresh tissue if extravasation of erythrocytes had occurred during life, as for example in a surgically removed hæmatoma. Avoidance of this deposit is of particular diagnostic importance in cases of malaria (Lendrum, 1944). Fortunately it is possible to give the fragile erythrocytes a protective primary fixation with formalin by 4-6 hours immersion, within which time formalin deposit has scarcely begun (Lendrum, 1941); they are then able to withstand the lytic action of the saturated aqueous mercuric chloride to which the tissue is transferred for further fixation. Their susceptibility to lysis by alcohol and water mixtures is not completely removed by the short formalin treatment.

The staining methods used in the past have been either fully differentiated fluorescein staining or the use of prolonged phosphotungstic-acid differentiation of acid fuchsin (C.I. 692); the former was often performed by overstaining with ethyl eosin (C.I. 770) followed by prolonged washing in water and alcohol; the latter by overstaining with an acidulated aqueous solution of acid fuchsin and subsequent differentiation in 1 per cent. phosphotungstic or phosphomolybdic acid. Both methods, however, show retention of the dye in muscle and, to a greater extent, in fibrin and the various acidophil granules. The phloxin-tartrazine method (Lendrum, 1947) is similar to the first scheme,

although if anything more effective, and has been successfully used in the study of the renal circulation (Trueta *et al.*, 1947). The picro-Biebrich variant of Masson's method as described below resembles the second scheme (especially if the nuclear staining be omitted) and has the advantage that under the conditions of the method as described, the red dye has a greater affinity for erythrocytes than for muscle, granules or fibrin. One can, with this method, obtain very clear demonstration of the distribution of erythrocytes by observation or photography through a red absorption (blue-green) filter.

One of the fascinations of Mallory's connective tissue stain lies in its susceptibility to modification. In the years since its introduction, endless variants have followed, most of them along the three main streams begun by Gallego, Heidenhain and Masson. The waywardness of Mallory's own method was to some extent responsible for its valuable progeny but unfortunately none of these inherited the virtue, particularly valuable to the pathologist, of staining fibrin a different colour from blood. The importance of this distinction in morbid anatomy led the writer to try to obtain this result under control and in the picro-Mallory methods (Lendrum and McFarlane, 1940 ; McFarlane, 1944) the pathologist has techniques of particular value for this purpose.

<h2 style="text-align:center">I</h2>

Recent work has shown that the specific staining of the fibrin is improved by the use of a strongly acidified fuchsin solution. A one per cent. aqueous solution of acid fuchsin containing 3 per cent. of trichloracetic acid has been found to give particularly good results in the method as detailed below. This solution gives, in addition, a clear red nuclear stain and can very well replace the fuchsin solution of the original Mallory method. Apparently acid fuchsin, which is a sulphonated dye, dissociates in an acid medium to give some free basic fuchsin and thus stains the nuclei. It is probable that at the time of the original Mallory methods there was enough unsulphonated fuchsin present in the acid fuchsin then available to give good nuclear staining. The greater purity of modern dyes may explain why recent workers have had to insert a separate nuclear stain into the original method, or have evolved variants giving special nuclear staining. This swing towards basic activity in acid solution provides a possible explanation of the variable results obtained with Mallory methods on the pituitary.

<h3 style="text-align:center">THE ACID PICRO-MALLORY METHOD</h3>

Paraffin sections to water.
Stain with celestin blue 3-7 minutes in jar.
Rinse in tap water.
Stain with Mayer's hæmalum 3-7 minutes.
Wash in tap water 2 minutes or longer.
Rinse in methylated spirit (95 per cent.).
Stain with picro-orange (0·2 per cent. orange G (C.I. 27) in 80 per cent. methylated spirit saturated with picric acid) 2 minutes.
Stain with 1 per cent. acid fuchsin in 3 per cent. aqueous trichloracetic acid 5 minutes.
Rinse in water.
Dip briefly in equal parts picro-orange and 80 per cent. methylated spirit.
Treat with 1 per cent. aqueous phosphotungstic acid at room temperature 5-10 minutes.
Rinse in water.
Stain with 2 per cent. soluble blue (C.I. 706) in 2 per cent. aqueous acetic acid 2-10 minutes.
Rinse in water.
Dehydrate with absolute alcohol, clear and mount.

Notes on the method

Preparation of celestin blue. Allow 2·5 g. of iron alum to dissolve overnight at room temperature in 50 ml. of distilled water : to this add 0·25 g. of celestin blue (C.I. 900) and boil for 3 minutes ; filter when cool into a staining jar and add 7 ml. of glycerol. This solution maintains its efficiency for several months.

Preparation of Mayer's hæmalum (1903 formula ; see Lendrum and McFarlane). Allow 1 g. of hæmatoxylin, 0·2 g. of sodium iodate and 50 g. of powdered potassium alum to dissolve overnight at room temperature in 1 litre of distilled water ; to this add 50 g. of chloral hydrate and 1 g. of citric acid and boil for 5 minutes. When cool it is ready for use. With some brands of hæmatoxylin it pays to double the content.

The combination of celestin blue followed by Mayer's hæmalum gives a nuclear stain which resists phosphotungstic or phosphomolybdic acid, and is distinctly less susceptible to aqueous picric acid than are most iron hæmatoxylins. This last property makes it a particularly valuable nuclear stain before Van Gieson's solution. The celestin blue jar lasts for months and the hæmalum is the routine solution which in a busy laboratory is always fresh. If both solutions are new, shorter staining times are advisable but better results can be obtained by differentiation, using 0·1 per cent. nitric acid, which, as Cole (1943) noted with iron hæmatoxylin, retains the black colour on the chromatin. The combined nuclear stain followed by nitric differentiation gives excellent chromatin staining. By using the combined nuclear staining in this method one darkens the nuclei and so throws up more vividly the pure red of the fibrin.

II

When the specific demonstration of fibrin is not important then the Masson type of variant is generally preferred, with its strong colour distinction between cytoplasm and collagen and its sharp black nuclear stain. The nuclear stain in Heidenhain's and Gallego's methods is less controllable than in Masson's, and in both, the red dye is apt to give an unpleasant refractile effect where the chromatin is closely aggregated. On the other hand, both the German and the Spanish method have an attractive general transparency often lacking in the French.

The method now to be described gives the clear nuclear staining of the Masson type and the transparency of the Heidenhain, with the further attribute of staining the erythrocytes in a brilliant and fairly selective way. Despite its apparent complexity, the method behaves consistently in different hands and the solutions keep well. Thus one can ask for the method at any time and be reasonably sure of a good result, given of course, good sections from well-fixed tissue.

Fixation. All the tissues used were fixed in formol-sublimate or formalin followed by saturated aqueous mercuric chloride (Lendrum, 1941).

THE PICRO-BIEBRICH METHOD

Paraffin sections to water.
Stain with celestin blue 3-7 minutes in jar.
Rinse in tap water.
Stain with Mayer's hæmalum 3-7 minutes.
Wash in tap water 2 minutes or longer.
Saturated aqueous picric acid 5 minutes.
Rinse in tap water.
Rinse in methylated spirit (95 per cent.).

Stain with picro-orange (0·2 per cent. orange G (C.I. 27) in 80 per cent. methylated spirit saturated with picric acid) 2 minutes.

Rinse in tap water.

Rinse in cellosolve.

Stain with Biebrich scarlet (C.I. 280) saturated solution in cellosolve 2-3 minutes.

Rinse in cellosolve.

Rinse in tap water.

Phosphotungstic acid 1 per cent. aqueous solution 5 minutes.

Rinse in water.

Stain with 2 per cent. soluble blue (C.I. 706) in 2 per cent. acetic acid 2-10 minutes.

Dehydrate with absolute methylated spirit.

Xylol.

Notes on the method

There appears to be an interaction between the picro-orange taken up by the erythrocytes and the Biebrich scarlet (or croceine scarlet, C.I. 286; or paper red, C.I. 252) if the red dye be in cellosolve; the results are much less specific with an aqueous solution. Further, with the method as given, the red dyes under the action of phosphotungstic acid come easily out of the collagen and thus the final blue in the collagen is pure and translucent. This last may be due in part to the picric acid, because a transparent blue is fairly characteristic of the interesting McFarlane variants of the basic picro-Mallory method.

It is not suggested that the picro-Biebrich method has a wide scope in the ordinary run of diagnostic histology, but any worker who has used the method will realise that for certain types of tissue it has a definite value. Certainly no single one of the Mallory variants is ideal for all purposes and it is part of histological skill to know the method which will prove most profitable in any special instance.

If the demonstration of muscle be the main reason for using a trichrome staining, one would reject the picro-Biebrich method along with Heidenhain's and Gallego's. In that case, one should use as first counterstain a dye with a greater affinity for muscle and the second counterstain should be one of low colour contrast. These desiderata are satisfactorily met in the lissamine red-tartrazine method (Lendrum, 1947, p. 452). This red dye, lissamine fast red BS, is a monazo dyestuff produced by coupling gamma acid on to acetyl meta-phenylene diamine sulphonic acid (I.C.I. Ltd.); it does not keep well in aqueous solution but a stock solution of 10 per cent. in 5 per cent. trichloracetic acid, which keeps for some months, can be diluted with water to give a 1 per cent. working solution.

III

Thus far, quite the most selective staining of erythrocytes in sections from paraffin, fixed as described above, has been obtained by staining with brilliant kiton red B (C.I. 748). This commercial dye, made by Ciba of Basle, Switzerland, was obtained through the courtesy of the Clayton Aniline Co. Ltd., Manchester. It dissolves readily in cellosolve to give a strongly coloured solution with some sediment, presumably inert salts, which can be ignored.

THE KRAG (KITON-RED ALMOND-GREEN) METHOD

Stain with Mayer's (1903-04, cited by Lendrum, 1947) hæmalum.

Differentiate if necessary, wash and blue.

Rinse with cellosolve from drop bottle.

Immerse in saturated solution of brilliant kiton red B in cellosolve, 20 minutes to 1 hour.

Differentiate in tap water.

Rinse with almond-green cellosolve solution.

Rinse briefly with cellosolve from drop bottle.

Replace cellosolve with xylol from drop bottle.

Mount in polystyrene (B.P.S., Kirkpatrick and Lendrum, 1941; Lendrum, 1947).

Notes on the kiton-red almond-green method

Any hæmalum can be used, but if photography of the erythrocytic distribution is intended, the nuclear stain should be light or omitted. The time of staining in the kiton red need not be very exact, but it is necessary to use the dye specified and to use it in cellosolve; an aqueous solution is ineffective. The almond-green solution is a mixture of saturated solutions in cellosolve of tartrazine (C.I. 640)* and of lissamine green BN 200 (I.C.I. Ltd.); the proportions are adjusted to give a yellow-green staining to all tissues other than the erythrocytes. This contrasts visually with the purplish red of the erythrocytes and a minus red (cyan) filter renders the erythrocytes quite opaque against a pale although recognisable background.

Muscle, fibrin, the granules of Paneth cells and the Russell bodies of plasma cells all lose the red on washing with water, pituitary granules are decolourised in the almond-green cellosolve solution, and almost the only materials other than erythrocytes retaining the red in this method are the acidophilic granules of the eosinophils. A few small kitonophil granules have been seen in one case in occasional epithelial cells of a major bronchus.

Incidentally, brilliant staining of the acidophils of the pituitary is obtained by using the cellosolve kiton-red solution as the first cytoplasmic stain in the Masson type of procedure :—nuclear staining with celestin blue and hæmalum, kiton-red staining, differentiation and fixation with phosphotungstic acid, second staining with light green.

SUMMARY

The principles of the fixation of erythrocytes in tissue are discussed, with special reference to post-mortem material.

Modifications of the Mallory connective tissue stain are considered and an improved method is given for the differentiation of fibrin from blood, the acid picro-Mallory method. Another variant is described, the picro-Biebrich method, which shows brilliant staining of erythrocytes along with a delicate trichromic background.

A new procedure, the Krag (kiton-red almond-green) method, is described for the selective staining of erythrocytes in sections from paraffin.

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* Tartrazine NS, a commercial dye made by Imperial Chemical Industries, is a very satisfactory product.

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578 . 67

ACID FIXATION (1) FOR THE PREVENTION OF ARTEFACTS AND (2) FOR DECALCIFICATION OF BONE

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One of the most annoying artefacts met with in histology is " pink disease," which especially affects formol-fixed biopsy specimens of lymphatic tissue and glandular epithelium. It gets its name from the appearance of the nuclei, which partly or wholly reject alum hæmatoxylin, and become acidophilic. If 2 parts of glacial acetic acid are added to 98 parts of 10 per cent. formalin this artefact does not occur. Acetic acid penetrates tissue rapidly and evenly, and seems to carry the formalin through the tissue with it. Unfortunately the specimen is often damaged by imperfect fixation before it reaches the laboratory, and the acetified formol is then of little value. If the de-paraffinised section is put into 1 per cent. hydrochloric acid in absolute alcohol for at least one hour before staining with alum hæmatoxylin, quite good nuclear staining results.

Since this indifferent nuclear staining with alum hæmatoxylin is not due to acid, it seemed worth while finding out if it is necessary to neutralise decalcified tissue. Pieces of formol-fixed bone were kept for different intervals of time in the acids commonly used for decalcification ; in each case better nuclear staining was obtained when the tissue was transferred to absolute alcohol without neutralisation than when it was neutralised before alcohol treatment. With certain acids the use of graded alcohols was detrimental. For practical purposes, 10 per cent. nitric acid in water provides a rapid and reliable decalcifying agent. It should be replaced daily, and the tissue removed immediately effervescence ceases. Nitric acid is a tissue fixative and hardens the tissue ; therefore nothing is gained from over decalcification, which often produces a yellow discolouration of the tissue and causes poor nuclear staining. This tissue discolouration is also produced when the temperature of the decalcifying fluid exceeds 70° F. The chief cause of tissue hardening is undoubtedly faulty dehydration, and to avoid this the final alcohol should be kept dry with anhydrous copper sulphate. Contrary to general opinion, perfectly dehydrated and cleared tissue can be left in molten wax for many days without becoming unduly hard or suffering distortion. Tissue normally requires at least 12 hours in molten wax for thorough impregnation. Giemsa diluted with 1 per cent. acetic acid provides a useful counterstain to Ehrlich's acid hæmatoxylin. Cartilage, newly formed bone and certain parasites difficult to demonstrate with hæmatoxylin and eosin are well defined by this method.

A simple method for decalcification and staining of bone

i. Fix slices of bone, not thicker than 3 mm., in 2 parts of glacial acetic acid and 98 parts of 10 per cent. formalin for at least 3 days.

ii. Decalcify with 10 per cent. nitric acid in water, changing the fluid daily and leaving the jar open. Remove tissue immediately effervescence ceases.

iii. Transfer tissue direct to absolute alcohol and dehydrate rapidly but thoroughly.

iv. De-alcoholise with chloroform for 12 hours and impregnate with molten paraffin wax.

v. Stain sections with Ehrlich's hæmatoxylin, differentiating rather longer than usual.

vi. Blue with tap water : rinse with distilled water.

vii. Counterstain for 10 minutes with :—

Giemsa	15 parts
1 per cent. glacial acetic acid . . .	10 ,,

For the sake of economy this should be done on the slide. Rinse stain off with distilled water and blot.

viii. Differentiate and dehydrate with absolute alcohol. Xylol. Mount in D.P.X.

The counterstain is controlled partly by the proportion of acetic acid, more acid providing stronger eosin staining, and the final alcohol, which extracts the blue stain. The method works well through a coating of celloidin. Any Romanowsky-type stain may be substituted for Giemsa, which was chosen because it is less liable to precipitate.

PERSISTENT VENOUS VALVES OF THE HEART

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(PLATE CV)

Persistence of the venous valves of the heart is not uncommon, but large Eustachian valves are seldom seen in the post-mortem room. In view of their association (and not infrequent confusion) in the literature with Chiari's network the following case is thought to be of sufficient interest to be recorded.

The Eustachian valve or valve of the inferior vena cava is usually represented in the adult heart by a muscular and membranous fold which extends from the region of the fossa ovalis along the sinus septum to the anterior part of the orifice of the inferior vena cava, where it disappears. It is seldom more than 1 cm. wide, and is usually entirely absent. The Thebesian valve lies below the Eustachian valve, and forms the valve of the coronary sinus; its variations are of little importance.

Description of specimen

The heart was that of a 48-year-old woman. The patient, a diabetic, was admitted to hospital *in extremis* and died a few hours later.

The heart weighed 360 g. The pericardium was normal, the myocardium pale and œdematous and there was some hypertrophy of the left ventricle. All the valves were normal and showed no changes apart from slight atheroma of the mitral. There was no ante-mortem thrombosis.

Through the orifice of the inferior vena cava a fenestrated membrane was seen extending over approximately 2/3rds of the lumen. The right auricle was opened by an incision through the orifice of the superior vena cava to a point ½ cm. anterior to the orifice of the inferior vena cava. This displayed a membranous Eustachian valve (fig.) attached to the inferior sinus septum, the anterior margin of the orifice of the inferior vena cava and the lower end of the crista terminalis. The free edge of this valve-like structure measured 4·5 cm. in length and was very lax. The maximum height of the valve was 2·5 cm. There were numerous fenestrations and in part the structure was made up only of a network of fine threads. The Thebesian valve was 1 cm. wide and divided the orifice of the coronary sinus into two parts ; it contained one fenestration. Both valves were made up of glistening white endocardium and contained no macroscopic muscle fibres. The foramen ovale was not patent and there were no other congenital abnormalities. There were no symptoms or signs discovered during life which could be referred to these structures.

Discussion

The structures described appear to represent an exaggeration of the normal vestigial Eustachian and Thebesian valves formed from the right venous valves. The attachments of the Eustachian valve are typical, extending along the inferior sinus septum upwards in front of the orifice of the inferior vena cava towards the crista terminalis. It is suggested that this valve demonstrates the site of a right venous valve which has undergone little retrogression, and that structures within this area, whatever their structure may be, represent remnants of the Eustachian valve and are not examples of Chiari's network.

Eustachi, in his *Opuscula Anatomica* (1563), gives an illustration of a heart with a Eustachian and a Thebesian valve. The Eustachian valve is partly membranous and partly fenestrated and is strikingly like the specimen described above. Von Rokitansky (1875, case 16) described a heart with anomalous membranes in the right atrium and fibres which Chiari (1897) later described in association with the Eustachian valve. This so-called " Chiari network " is made up of fibres in the right atrium extending from the Eustachian or Thebesian valves, or the region of the orifices of the inferior vena cava or coronary sinus, to the upper portions of the crista terminalis, inter-atrial wall or tricuspid valve.

In a review of the literature on venous valves of the right atrium of the heart, Yater (1929) described 11 cases, in 4 of which there was a Chiari network. He stresses that a true Chiari network is made up of fibres with anomalous insertions outside the possible limits of the right valve of the sinus venosus. Networks of the Eustachian or the Thebesian valves are not uncommon, and may be represented by a few fibres only. However, these fibres should be called remnants of the venous valves if they are within the territory of such valves, and should only be called Chiari networks if their insertions are anomalous. It is necessary to assume that a Chiari network is formed by the fœtal valve having anomalous attachments, and that during retrogression of the valve these attachments are drawn out into fine threads. During fœtal life the right valve of the sinus venosus is almost large enough to divide the right atrium

PLATE CV

PERSISTENT VENOUS VALVES OF THE HEART

Photograph of the right side of the heart showing the Eustachian and Thebesian valves. The Eustachian valve appears as a much fenestrated membrane in the upper left-hand quadrant; the Thebesian valve lies immediately below and to the right.

into two chambers; it is therefore possible that in some cases the free edge of the valve may become attached to unusual parts of the atrial wall.

It is agreed that the Eustachian and Thebesian valves are derived from the right valve of the sinus venosus. The origin of Chiari's network was thought by Chiari himself to be from the septum spurium as well as the right venous valve, while Jordan (1926) thought the left valve also played a part. Yater considered that the right venous valve only was involved. The conception of venous valves and Chiari's network being the same structure which has undergone changes due to different attachments makes it necessary to believe that Chiari's network is also derived from the right venous valve.

It was thought that during foetal life the Eustachian valve served to direct the blood from the inferior vena cava through the foramen ovale into the left auricle, and that with the termination of the foetal circulation the Eustachian valve regressed. Franklin (1948) considered that the Eustachian valve persists only in hearts in which an element of the blood flow from the superior vena cava is directed towards the orifice of the inferior vena cava and he has been able to correlate the height of the Eustachian valve with the direction of this blood-flow. The Eustachian valve recorded above is larger than those described in Franklin's work and is of interest in that the blood-flow from the superior vena cava appears to be partly directed towards the floor of the Eustachian valve. It would seem that this has caused the valve to persist.

Summary

A heart with persistent venous valves is described. The origin and site of these valves and the association of Chiari's network with the Eustachian valve are discussed. The persistence of these valves is thought to be due to a variation in the direction of blood-flow from the superior vena cava.

My thanks are due to Professor G. R. Cameron, F.R.S., who performed the post-mortem, for much help and encouragement.

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GLYCOGEN IN THE COLLECTING TUBULES OF NEW-BORN ANIMALS

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(PLATES CVI AND CVII)

When studying the glomerular basement membrane of the new-born rabbit, some sections were stained by the periodic acid-Schiff technique (McManus, 1948). In these preparations attention was immediately drawn to numerous conspicuous purple-red to magenta granules of various sizes, filling the cells of the collecting tubules.

The periodic acid-Schiff technique is a specific histochemical test for a large class of polysaccharides, the periodic acid converting the carbohydrate to an aldehyde which subsequently re-colourises the Schiff reagent, as in the Feulgen reaction. The polysaccharide most likely to be responsible for the intense specific staining of these granules in the collecting tubules appeared to be glycogen, and proof that this was the case was obtained by a positive result with Best's carmine and a negative reaction with both methods after salivary digestion.

Without exception in all the new-born rabbit kidneys examined—about 30—the collecting tubular epithelium was so loaded with glycogen that, in periodic acid-Schiff preparations, the tubules could easily be traced from their mouth on the papillary surface to their peripheral termination beneath the capsule, the deposits stopping short at the junction of the collecting tubules with the as-yet undifferentiated youngest generation of renal vesicles (fig. 1).

Since no previous reference to the presence of glycogen in this site could be found and in order to exclude its being a peculiarity of the new-born rabbit, the kidneys of the ageing rabbit and of three other species, guinea-pig, rat and man, both new-born and adult, were examined.

Glycogen was found in the collecting tubular epithelium of all the new-born animals, though the amount varied with the species. In contrast, after the neonatal period, glycogen was either absent or, if present, only in very small amount—a mere fraction of that in the new-born animal.

These histological findings were confirmed by a quantitative analysis of the glycogen in the kidney of the young rabbit at various periods after birth kindly carried out by Prof. McCance of the department of experimental medicine, Cambridge (table).

TABLE

Glycogen content of the kidney of the young rabbit at various periods after birth

No.	Age in days	Kidney		Liver	
		Weight (g.)	Glycogen (per cent.)	Weight (g.)	Glycogen (per cent.)
1	1	0·15	0·51	1·44	4·0
2	1	0·18	0·54	1·83	4·4
3	1	0·12	0·66	...	3·1
4	7	0·60	0·31	2·99	4·1
5	7	0·63	0·29	4·57	4·1
6	14	1·29	0·22	6·6	5·9
7	14	1·12	0·29	6·3	5·5
8	21	2·1	0·14	9·9	8·7

The epithelium of the renal pelvis and the ureter of all these animals also contained glycogen, but the amount was very variable and did not show any constant relation either to the age of the animal or to the quantity in the collecting tubule. In no instance was glycogen ever demonstrated in any other portion of the nephron than that derived from the metanephric duct.

Of the four species examined, the collecting tubules of the new-born rabbit contained the most abundant deposits of glycogen. After the third week, however, the picture was quite altered and only traces were left of this large store (compare figs. 2 and 4 with figs. 3 and 5). It was, moreover, less widely distributed throughout the tubule, being almost entirely confined to the mouth of the duct and to a length of tubule lying at the junction of the inner and outer medullary zones.

PLATE CVI

GLYCOGEN IN KIDNEY OF NEW-BORN

FIG. 1. - Kidney of new-born rabbit. Collecting tubules made conspicuous by their glycogen content in a periodic acid-Schiff preparation. ×35.

FIG. 2.—Kidney of new-born rabbit : junction of middle and external medullary zones. Cells of collecting tubules loaded with glycogen. Periodic acid-Schiff. ×75.
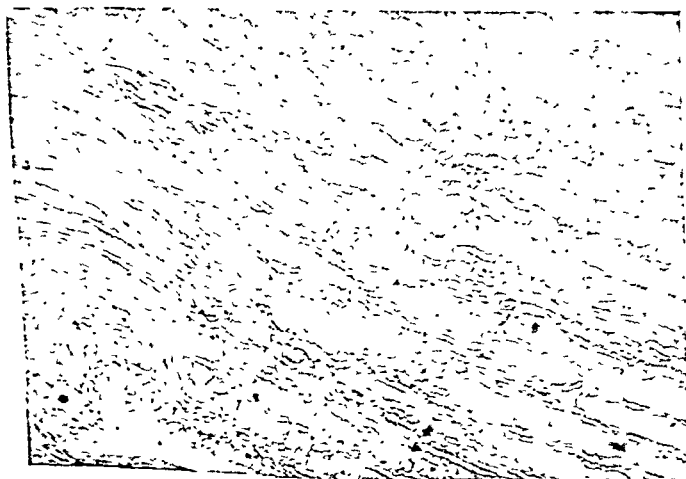
FIG. 3.—Kidney of 6-weeks-old rabbit : same region as fig. 2. Glycogen not present in collecting tubules. Periodic acid-Schiff. ×75.

GLYCOGEN IN KIDNEY OF NEW-BORN
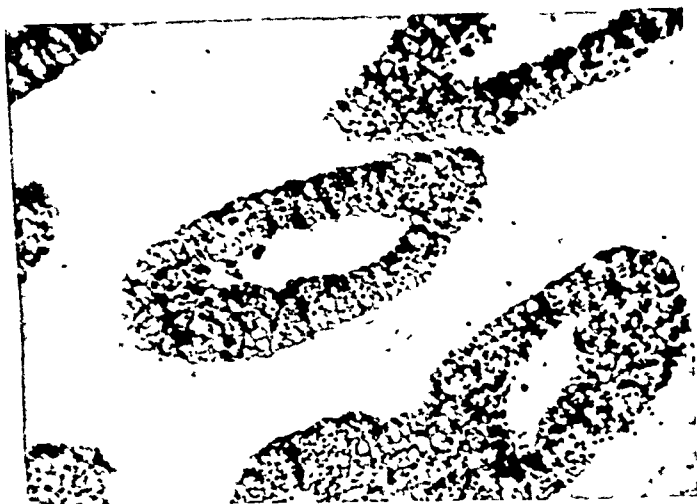
FIG. 4 —Kidney of new-born rabbit: same region as fig. 2. Periodic acid Schiff. ×300.

FIG 5 —Kidney of 6 weeks old rabbit: same region and magnification as fig 4 Small deposit of glycogen in collecting tubules at junction of middle and external medullary zones None was present elsewhere in these tubules Periodic acid Schiff ×300.

FIG. 6 —Kidney of new-born rabbit. Differentiated bar of cytoplasm on luminal border of cells lining collecting tubules. Heidenhain's iron hæmatoxylin ×1600.

In the new-born guinea-pig the glycogen content was much more variable, and although in some cases almost as abundant as in the new-born rabbit, in others it was comparable with that in the adult rabbit; while in the adult guinea-pig none was ever demonstrated. Similarly in the rat, the moderate deposits in the new-born animal disappear in the adult. Observations on human autopsy material were limited by the poor preservation of the collecting tubular epithelium; but a considerable amount was found in one relatively well-preserved kidney from a new-born infant, while the only glycogen demonstrated in an adult was a minute trace at the mouth of a collecting duct in a kidney removed from a 35-year-old man.

From these results it may be concluded that in some species at birth the collecting tubular epithelium may contain significant and sometimes even abundant deposits of glycogen, which, in the older animal, disappear or are very greatly reduced.

It is hoped to investigate the physiological role of these stores of glycogen—whether they are mobile and responsive to extra-renal stimuli, thus resembling liver glycogen, or, like muscle glycogen, are concerned only with local function and uninfluenced by the general metabolic activity of the animal. Their relation, if any, to renal function is still only a matter of speculation. The work of McCance (1948) and others has shown, however, that the function of the new-born kidney differs from that of the adult and that a relatively abrupt transition occurs soon after birth, and it is tempting to correlate these neonatal glycogen stores with the specialised metabolism of the same period. This indeed may explain the more variable picture presented by the new-born guinea-pig. This animal is much more mature at birth than the rabbit (60 days gestation compared with 30), and it may be that the change over from the infantile type of renal metabolism is already taking place in those new-born animals whose tubules show varying degrees of glycogen depletion.

Another observation was made on the same material which, although not strictly relevant, is of interest as probably bearing on the functional activity of the cells: it does not appear to have been described before. This was the presence of a narrow bar of differentiated cytoplasm along the convex free border of these same cells lining the collecting tubules (fig. 6). It was most conspicuous in the young rabbit, but could be recognised in the other species and in the adult. There was no apparent relation to the glycogen content of the cell, but alkaline phosphatase preparations suggested that in some cells it might be the site of enhanced enzyme activity. It is probably comparable to the striated border of the proximal convoluted tubules and the intestinal epithelium and may be concerned in maintaining the composition of the urine constant in its passage to the exterior.

Summary

1. Glycogen has been demonstrated in the collecting tubular epithelium of four species of new-born animals (rabbit, guinea-pig, rat and man), using the periodic acid-Schiff technique. In the adult it is either absent from this site or present in only very small amounts.

2. A differentiated bar of cytoplasm along the luminal border of the cells lining the collecting tubules is also described. It does not appear to have any relation to the glycogen content of the cell.

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SOME TUMOURS FOUND IN WHALES

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(PLATES CVIII AND CIX)

Through the courtesy of the " Discovery " Committee we have inspected the large collection of material from whales obtained by observers in the Antarctic oceans and preserved at the British Museum (Natural History), South Kensington. Of this collection, only a few specimens had been set aside as abnormal. Among these were a number of calcified parasitic cysts from various sites, including an adult female worm full of ova, which appeared to be the source of a large, branched and calcified thrombus in the inferior vena cava of a fin whale. There were also a number of firm smooth masses which had been found attached to the pleura of several different species. On section, these all proved to be of a chronic inflammatory nature and parasites may well have been responsible for their origin. Our main interest was in the following six tumours.

Uterine fibromyoma

From whale no. 144 ; species and date of collection not stated. The tumour, which was said to have been found near one cornu of the uterus, consisted of an ovoid, well-circumscribed mass 4 cm. in longest diameter, attached by a short pedicle to a piece of more spongy tissue. Microscopically, the mass has the typical structure of a benign fibromyoma and resembles the tumours found in the human subject. The attached tissue is normal endometrium.

Papilloma of tongue

This specimen came from an unnumbered blue whale collected in January 1929. Macroscopically it consisted of a pedunculated mass about 8 cm. in diameter, with a coarsely papillary surface and some pigmentation in the deeper part of the thick epithelial zone. Microscopically, the tumour shows the typical structure of a benign squamous-celled papilloma (figs. 1 and 2), the only unusual feature being that the pigmented dendritic cells of the deeper layers of the epithelium are particularly prominent (fig. 3).

Mucinous cystadenoma of ovary

This specimen was from a blue whale (no. 117) approximately eighty feet in length, caught in March 1948. It consisted of one-half of a multiloculate cystic tumour about 12 in. across. The superficial part of the mass consisted of a layer of fibrous ovarian tissue, the central part of a number of quite small cysts, devoid of hæmorrhage, containing gelatinous fluid and radially arranged around a central yellow core. The tissue was not abundant between the cysts, which in many places were separated only by thin, translucent, fibrous walls. Sections from the periphery of the mass show normal ovarian tissue with typical ovarian stroma and some corpora albicantia, but no maturing follicles or corpora lutea, structures which are very large in whales. Sections of the cystic area show the tumour spaces to be lined partly by columnar mucus-

PLATE CVIII

TUMOURS OF WHALES

FIG. 1.—Papilloma of tongue.   ×4.

FIG. 2.—Papilloma of tongue.   ×35.

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TUMOURS OF WHALES
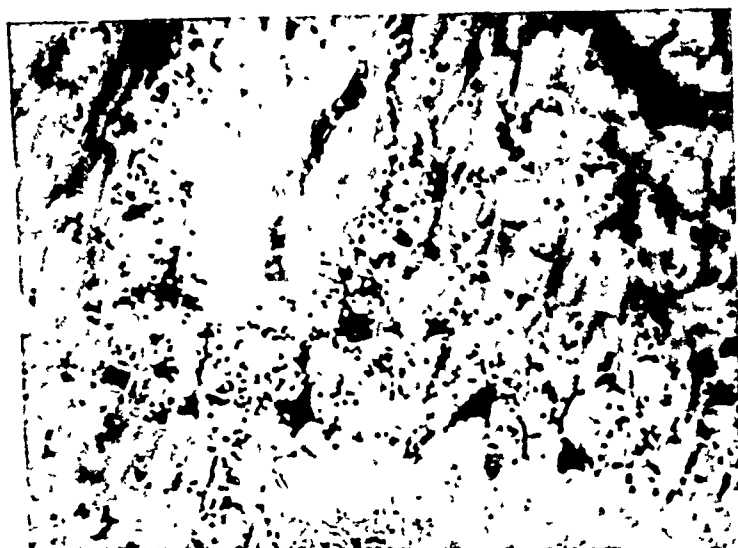
FIG. 3 —Dendritic pigment cells in epithelium of papilloma of tongue. ×470.

FIG. 4 —Mucinous cystadenoma of ovary
of blue whale ×70.
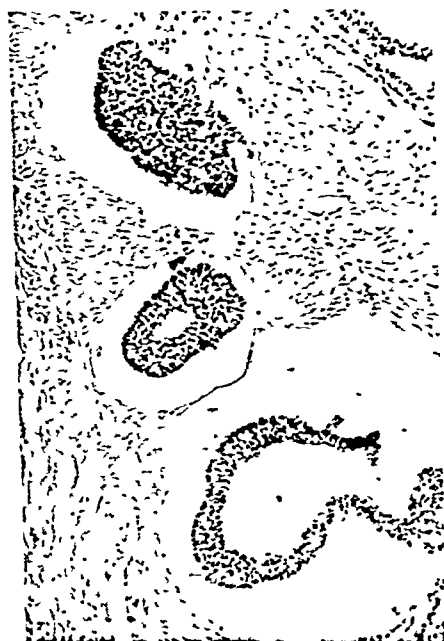
FIG. 5 —Granulosa-cell tumour of ovary
of fin whale. ×70.

secreting epithelium, partly by irregularly stratified polygonal-celled epithelium, the former sometimes lying superficial to the latter (fig. 4). Sections of the yellow central core show only degenerated tissue.

Granulosa-cell tumours of ovary

Three tumours were found which could be classed as of this type.

The first was from a pregnant blue whale (no. W/2612), approximately eighty feet long, killed near South Georgia in October 1929. A " cyst " had been found in one ovary and removed and fixed separately from the rest of the organ. The observer had evidently not considered it to be a corpus luteum. The mass was round, 3 cm. across and had a fibrous capsule. On section a papillary epithelial tumour is revealed which contains many small cystic spaces. Most of these appear empty, but a few contain eosinophilic colloid material. The main point of interest, however, is in the lining of these cysts, which is composed of granulosa cells. In the larger cysts these are flattened out into a single layer, but in the smaller they are several layers thick, while in a few places the contained space is very small. The relative sizes of the granulosa-cell layer and cyst cavity are never such as to produce the " rosette " appearance so often seen in these tumours in smaller animals. The granulosa cells are all rather degenerate. As is usual in granulosa-cell tumours, theca cells are also evident in the stroma under the lining of the cysts and in places form definite sprouts beneath it.

The second example was from a female fin whale (no. 1745), caught in an unnamed locality in March 1928 ; length not stated. The specimen was described as a " large follicle from ovary containing a solid body " and consisted of part of the fibrous wall of a cyst. Projecting from the surface was an irregular nodule 3·5 cm. across consisting of small cysts which contained mucoid material and a mass of calcified material or bone. On section (fig. 5) cysts and stroma have a similar structure to the previous tumour, but the granulosa cells are less degenerate, the thecal cells less active and there are areas of calcification.

The third example was from a pregnant fin whale (no. 2615) caught off South Georgia in the whaling season of 1937-38. She was about sixty-five feet long. The specimen, described as a " fibrous cyst," consisted of two pieces of well-circumscribed tumour covered with peritoneum and containing many small cysts and much fibrous tissue. Its original size and shape were not noted. Microscopically the cysts are lined with granulosa cells, but these are much more irregular in their arrangement than in the previous specimens. The intervening stroma consists of dense fibrous tissue.

DISCUSSION

The two species of whale mentioned, the fin whale (*Balænoptera physalus*) and the blue whale (*B. musculus*), are the only ones now of commercial importance, as they are much the largest and give much the highest yield of edible oil. Sperm whales are also large, but as their oil does not mix with that of the blue or fin whale, they are seldom captured. During the 1947-48 whaling season, the average length of blue whales captured was 85 feet, while the fin whales ranged between 68 and 90 feet. However, the blue whale can attain a length of as much as 100 feet, possibly more, and is certainly the largest vertebrate known ever to have existed. The semi-aquatic giant reptiles, *e.g.* Diplodocus, never attained this length and their relative bulk was also much less. It is evident, therefore, that the tumours described were very small as compared with the bulk of these gigantic creatures, especially the papilloma of the tongue, an organ which is relatively larger in the whale than in most animals.

There is no doubt as to the nature of the uterine fibromyoma, the papilloma of the tongue or the ovarian mucinous cystadenoma, but the granulosa-cell tumours call for comment. The ovaries of whales show no features notably different histologically from other mammals except the very large size of their corpora lutea. The three specimens examined could not be interpreted as due to simple follicular or luteal cystic change or to atresia, and their appearance so closely resembles that of other mammalian granulosa-cell tumours that we have no hesitation in calling them by this name. The fact that 3 out of 6 tumours collected haphazard were of this kind suggests that they may be common in whales. On the other hand, the sample is a small one, as the " Discovery " observers see only a small proportion of the whales captured each season, and in any case are not primarily interested in pathological conditions. Naturally we have no evidence about any possible endocrine disturbances in whales with these tumours.

SUMMARY

A papilloma of the tongue, a fibromyoma of the uterus, a mucinous cyst-adenoma of the ovary and three granulosa-cell tumours of the ovary in either the blue whale or the fin whale are described. Their structure is similar to that of tumours of these types observed in other mammals. We do not know of any previous reports of tumours in whales.

616 . 15—097 . 34—02 : 546 . 155—33

ANTIGENIC MODIFICATION OF HUMAN RED CELLS BY PERIODATE

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Treatment with periodate has been shown to be capable of destroying the influenza virus receptors of fowl and human red cells (Hirst, 1948 ; Hotchkiss, cited by Hirst ; Burnet, personal communication to Briody, 1948) and of rendering human red cells panagglutinable (Burnet—Briody, *op. cit.*). Panagglutinability and receptor destruction also occur in human cells treated with influenza virus or with *V. cholerœ* filtrate (Burnet et al., 1946 ; Stone, 1947).

The present communication reports some observations which demonstrate the acquisition by periodate-treated human red cells of a new antigenic specificity. This antigenic change appears to be distinct from that which occurs in cells treated with a Vibrio preparation.

In the preparation of suspensions for hæmagglutination tests the following techniques are employed. Periodate treatment is carried out by allowing equal volumes of a 50 per cent. washed group-O red-cell suspension and M/100 potassium periodate in saline to react for half-an-hour at room temperature, after which the cells are washed in saline. Treated cells undergo hæmolysis, but by carrying out the tests without delay the degree of hæmolysis is not sufficient to interfere with the interpretation of results. Though periodate treatment has a tendency to produce some degree of cell instability, the

suspensions obtained are as a rule satisfactory for the performance of agglutination tests. For Vibrio transformation, equal volumes of a 50 per cent. cell suspension and the supernatant obtained by centrifugation or by sedimentation in the refrigerator of a broth culture of Vibrio sp. (N.C.T.C. 4711) are incubated at 37° C. for 1 or 1¼ hours, the cells being then washed in saline. Agglutination tests are carried out by mixing in tubes equal volumes of a 5 per cent. cell suspension and serum or dilutions of serum. The tubes are allowed to stand at room temperature for half-an-hour and the results are then read by examination of the sediment with a hand lens.

In the accompanying table are shown the titres of the serum of a rabbit before and after immunisation with periodate-treated cells, and of an immune rabbit serum prepared against Vibrio-treated cells. The specific immune

TABLE

Titration of hæmagglutins in rabbit sera for group-O red cells

Rabbit sera	Red cells used for absorption	Test cells		
		Untreated	Periodate-treated	Vibrio-treated
R 118 before immunisation with periodate-treated cells	None	<20	<20	40
	Untreated cells	<20	<20	<20
R 118 after immunisation with periodate-treated cells	None	<20	5120	40
	Untreated cells	<20	5120	20
	Vibrio-treated cells	<20	5120	<20
R 8 after immunisation with Vibrio-treated cells	Untreated cells and periodate-treated cells	<20	<20	320

Titres are expressed as reciprocals of the final serum dilution

agglutinins in the sera of these two rabbits were each capable of absorption by the homologous treated cells. The sera were titrated against normal cells and also against cells treated with periodate and with the Vibrio preparation. It will be seen that periodate treatment affects an antigenic modification of the cell capable of stimulating the production of a specific antibody in the rabbit. The titre of serum R 118 against Vibrio-treated cells after immunisation does not represent a significant increase on the pre-immunisation level. Thus it would appear likely that periodate treatment effects not only a specific change in the cell but also that the changes occurring in periodate-treated and in Vibrio-treated cells have no common antigenic feature. A further point illustrated by these results is the failure of periodate-treated cells to produce significant antibody against normal cells. This latter finding would suggest a destructive action of periodate on the immunising property of the antigen responsible for inducing antibody production against normal cells.

By cross-absorption tests with normal—*i.e.* non-immune—human serum it has also been found that the panagglutinin for periodate-treated cells is distinct from the panagglutinin for Vibrio-treated cells ; neither agglutinin is removed by absorption with normal cells. It is presumed that these agglutinins in normal human serum are specific for the acquired antigenic character demonstrated by immune rabbit sera.

Summary

Human group-O red cells, when treated with potassium periodate, develop a new antigenic specificity which is distinct from that occurring in cells treated with a Vibrio preparation.   Periodate-treated cells are susceptible to a pan-agglutinin present in human serum; this panagglutinin is distinct from the panagglutinin in serum (? T agglutinin) for Vibrio-treated cells.

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616 . 131 . 3—007 . 22—007 . 64

ANEURYSM OF THE DUCTUS ARTERIOSUS

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(PLATE CX)

CASE REPORT

Clinical history

The patient was an 18-days-old breast-fed female child, admitted to hospital because of an abscess in the skin of the right upper arm which had appeared six days previously, a more recent inflammatory swelling in the right leg and proptosis of the left eye of two days' duration.   The child was still below birth weight.   On examination, the arm abscess, which had been incised, was healing. The right leg was diffusely swollen and reddened and it pitted on pressure. T. 98, P. 110, R. 80.   The respirations were of a grunting character.   Percussion note and air entry were diminished at the right base.   There was marked proptosis of the left eye, the lids of which were reddened and swollen.   Coagulase-positive hæmolytic *Staphylococcus aureus* was grown from the arm abscess.   W.B.C. 10,600 per c.mm. (neutrophils 88 per cent., mostly band forms and myelocytes; lymphocytes 12 per cent.).   The child was given large doses of penicillin intra-muscularly, but the temperature, pulse and respiration rose precipitously and death supervened 12 hours later.

Autopsy

The heart weighed 19 g.   There was a small deficiency in the anterior portion of the membrane covering the foramen ovale, otherwise no abnormality could be detected.   The ductus arteriosus was the seat of a fusiform aneurysm (figs. 1 and 2).   The aortic opening, 0·5 cm. in diameter, was 2·5 cm. from the cusps of the aortic valve.   It lay just distal to the point of origin of the left subclavian artery and just proximal to the apertures of the first intercostal arteries.   The pulmonary opening, 0·2 cm. in diameter, was 1·5 cm. from the cusps of the pulmonary valve.   It lay just inferior to the first part of the right

FIG. 1.—Aneurysm of ductus arteriosus: oblique view. A, aorta; B, pulmonary artery. A window has been cut into the aorta at the point of entry of the ductus. Very slightly enlarged.

FIG. 2.—Aneurysm of ductus arteriosus: lateral view. A, aorta; B, pulmonary artery. Very slightly enlarged.
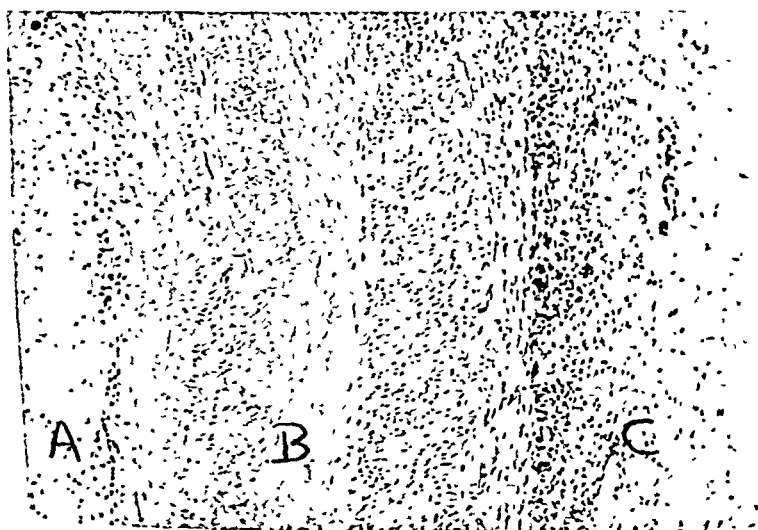
FIG. 3.—Photomicrograph of wall of aneurysm. A, portion of inner necrotic zone; B, outer portion of media showing extension of necrosis; C, adventitia. ×90.

branch of that artery. The ductus was 1·5 cm. in length and its maximum diameter was 0·9 cm. On section the wall was approximately 0·1 cm. thick and was of a mottled brown colour. The lumen contained blood clot.

Summary of other findings. There was an incised abscess in the skin of the right upper arm. Bronchopneumonia and lung abscesses were present in both lungs and there was extensive fibrino-purulent pleurisy. A small abscess was present in the anterior mediastinum, cultures from which yielded *Staphylococcus aureus*. The abdominal viscera showed no gross abnormality. Pus was present in the retro-orbital tissues on the left side. The bone in the region of the optic foramen had undergone necrosis and pus had extended to the extradural position internal to this. No thrombus was present in the cavernous sinus. The meninges were congested but the brain itself showed no abnormality.

Histology

Sections were stained with hæmatoxylin and eosin and hæmatoxylin and Van Gieson, and with Gram's stain. The inner quarter of the wall, which appeared to consist mainly of media, was almost entirely necrotic. The rest of the media was degenerate and contained irregular extensions of the necrotic change from the inner portion (fig. 3). There was no increase of fibrous tissue in this layer. At one point the intimal endothelium survived. No internal elastic lamina could be identified. The thrombus present in the lumen appeared to be firmly adherent to the necrotic inner layer. There was round-cell infiltration of the adventitia, the cells being for the most part histiocytic, though a moderate number of lymphocytes were also present. Occasional eosinophil cells were present. The cellular infiltration appeared most intense where the necrosis of the media was most advanced. The vasa vasorum showed no obvious abnormality. Gram-stained sections revealed no organisms.

Discussion

Aneurysm of the ductus arteriosus has been recorded but rarely. Kaufmann (1929) quotes Simmonds's view that some of the earlier cases were not true aneurysms of this vessel but aortic aneurysms in the region where the ductus enters the aorta. There is a diagram in Kaufmann's book of an aneurysm of this vessel which occurred in an eleven-day-old girl who died of sepsis arising from umbilical phlebitis. Apart from the extension of the contained thrombus into the lumen of the aorta which took place in his case, the similarity, both anatomically and clinically, to the present instance is remarkable. Taussig (1947) mentions four cases of aneurysm reported in the literature but does not include Kaufmann's case. According to her, the formation of a fusiform aneurysm will only occur when the obliteration of the ductus is complete at the pulmonary end, but incomplete at its point of entry into the aorta. In this case, the pulmonary end was greatly stenosed, though apparently not occluded, while the aortic aperture was widely patent. Taussig states that the earlier obliteration of the pulmonary end is what normally happens in the closure of the ductus. The degeneration of the media was probably the cause of the distension of the remainder of the vessel by the blood propelled into it from the aorta. The thrombus formation appeared to be secondary to the necrosis of the inner part of the wall. The cause of the necrosis is not clear. Blumenthal (1947) has shown that it takes place sometimes in a ductus undergoing normal involution. He expresses the view that degeneration of the wall starts from about the twelfth day after birth, as a result of anoxia. If this is slight, the usual sequence is a gradual replacement of muscle tissue by tissue of a lower order, but if it is more severe necrosis results. The anoxia is postulated as being secondary to active contraction of the muscular wall which takes place at birth. This contraction does in fact take place in the experimental animal.

and the increase in the oxygen content of the blood occurring with the onset of respiration has been shown to be the probable physiological factor initiating this contraction (Kennedy, 1942). If this view is accepted, the sequence of events in the present case would be an initial prolonged contraction of the vessel at birth, followed by the relaxation of all but the pulmonary end, in which fibrous replacement of muscle may have begun. The relaxed portion is damaged by the anoxia arising from the contraction, and distends under the blood pressure maintained through the aortic orifice. A thrombus subsequently forms, and it is probable that, had not death supervened, the end result through organisation would have been much the same as if no aneurysm had formed.

The coincidence that both this case and that of Kaufmann were subjects of neonatal sepsis raises the question of an infective factor. In the present case, no lesion was found in the wall of the ductus which could be attributed definitely to bacterial inflammation. The adventitia showed infiltration with histiocytes and lymphocytes, but it is reasonable to suppose that these cells were present as a reaction to the necrosis which had occurred in the vessel wall.

SUMMARY

1. A case showing an aneurysm of the ductus arteriosus—a rare anomaly—is reported.

2. The suggestion is made that the aneurysm formed in consequence of prolonged physiological contraction of the vessel wall at birth. With the occurrence of anoxic necrosis of the central portion and replacement fibrosis at the pulmonary end, blood propelled in from the aorta could bring about the dilatation observed.

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AN UNUSUAL CASE OF URINARY TRACT AND CARDIAC ABNORMALITIES

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(PLATE CXI)

The combination of congenital abnormalities of the urinary tract found in the case here reported is not mentioned by any of the following authorities :— Beer and Hyman (1930), Campbell (1937), Mitchell (1945). The case has therefore been considered worthy of record.

Case history

E. H. was 5 weeks old when admitted to this hospital on 19.5.48. He had been breast-fed from birth but had never gained weight well. Twelve days earlier (7.5.48) he had been admitted to another hospital with a diagnosis of " underfeeding." He was discharged from there on 14.5.48. At this stage the mother's milk failed entirely and the baby was given an artificial food in adequate amounts, but he did not take feeds well and lost weight. The mother is well known to the psychiatry department, who gave a bad report on her character and general conduct.

The child was blue at birth and weighed 7 lb. 2 oz. On examination, temperature, pulse and respirations were normal. The weight was 6 lb. 1 oz. (expected weight 8 lb. 11 oz.). A marasmic dehydrated infant. Heart not enlarged ; systolic murmur, maximal in 4th left intercostal space. Lungs, abdomen and central nervous system normal. Right talipes equino-varus.

Dehydration was rapidly corrected by subcutaneous saline and the weight rose to 6½ lb., but he still took feeds badly. Urine was passed in apparently normal amount. Three days after admission there was a bout, lasting half-an-hour, of twitching of the head and limbs, with cyanosis of the lips. Two days later there was another similar bout, shortly after which he died (24.5.48).

Post-mortem findings

The post-mortem was made 12 hours after death.

External. A wasted male infant with sunken eyes and depressed fontanelle ; right talipes equino-varus.

Circulatory system. Right heart dilated. Myocardium pale. No valvular or septal defects. Ductus arteriosus arising from right pulmonary artery at a point corresponding to the usual point of origin from the left pulmonary artery and attached to the arch of the aorta at the usual place. The ductus was not patent ; the tip of a probe would enter its aortic end, but the other end would not accept even that. There were no other abnormalities of the great vessels in this region.

Respiratory system. Lungs rather pale, with dark red or brown spots about 2·0 mm. in diameter scattered throughout their substance.

Alimentary system. No abnormality found.

Haemopoietic and endocrine systems. Bone marrow very pale.

Urogenital system. The left kidney (26 g.) showed considerable pelvic hydronephrosis with infection ; there were numerous small abscesses throughout the

cortex. There was also marked hydro-ureter (fig. 1). A probe passed freely up the ureter as far as the pelvi-ureteric junction, but there was no stenosis here, for urine could be " milked " freely from the pelvis into the ureter, and 30 per cent. sodium iodide, injected by syringe and cannula, passed freely from ureter to pelvis (fig. 2). The uretero-vesical opening was normal.

The *right kidney* (12·5 g.) was a mass of small cysts ; no kidney tissue could be seen. The cysts varied widely in size, the biggest being 1·5 cm. in diameter ; they were filled with clear straw-coloured fluid. There was no visible renal pelvis ; in its place was a thread-like structure 2·0 cm. long, the lower end of which was continuous with the right ureter (fig. 1). This ureter was not distended ; its lumen stopped at the junction with the thread-like structure, as far as could be determined by probe and by the injection of sodium iodide followed by radiography (fig. 2). The uretero-vesical opening was normal.

The *bladder* was contracted and apparently normal. No urethral valve was found.

Nervous system. Nothing abnormal found.

Osseous system. Only three centres of ossification in the body of the sternum.

Urea estimations were performed as follows :—

Cerebrospinal fluid	411 mg./100 ml.
Fluid from cysts of right kidney . .	405 mg./100 ml.
Urine from pelvis of left kidney . .	504 mg./100 ml.

Histology

Left kidney. Apparently this was originally of normal structure, but now shows the usual changes of ascending pyelonephritis.

Right kidney. Most of the cysts have a wall of fibrous tissue and a lining of flattened or cubical epithelium. Some cysts, both small and large, have in their walls a variable amount of smooth muscle, mainly arranged circumferentially. Embedded in the fibrous tissue between the cysts are a few islands of atypical tubules and glomeruli.

Lungs. Small foci of collapse.

Discussion

Urinary tract abnormalities are not uncommon in children. Mitchell states that the recorded incidence varies between 5 and 12 per cent. ; while Bigler (1929) reports an incidence of 13 per cent., with other congenital abnormalities in about half the cases. The individual lesions in the present instance are not uncommon, though their combination in one case is apparently unique.

The polycystic kidney. According to Sieber (1905) as cited by Campbell (1937), polycystic disease of the kidneys is unilateral in less than 10 per cent. of cases. In fact Sieber collected from the literature only cases over 20 years of age. There were 212 of these and only 9 were unilateral—6 left, 3 right. Sieber says also that where the condition is bilateral it is usually more advanced on the left side than on the right.

Campbell himself, in 12,080 autopsies on children, found the condition to be one-sided in 20 out of 48 cases. Of these 48 cases 35 died before the age of 6 months, but it is more usual for the disease to become manifest in middle life with the onset of renal failure. Campbell considered that the condition might have developed on the other side in his unilateral cases if they had lived longer. In 7 of his cases of polycystic disease there was also a congenital stricture of the ureter and in 4 there was idiopathic dilatation of the ureter, but these two ureteric abnormalities were never present in the same case.

The genesis of polycystic disease is usually attributed to a failure of fusion between the down-growing metanephric uriniferous tubules and the upgrowing

PLATE CXI

J. PATH. BACT.—Vol. LXI

URINARY AND CARDIAC ABNORMALITIES

Fig. 2.—Radiograph of kidneys, ureters and bladder after retrograde injection of sodium iodide. ×0·8.

Fig. 1.—Kidneys, ureters and bladder seen from the front. ×0·8.

tubules sprouting from the ureteric bud. Thus many " undrained " uriniferous tubules become distended by their own secretion ; but a minority of tubules develop normally and carry out their normal function. It seems likely that the muscle-walled cysts in the present case are really renal calyces distended by urine formed by " drained " nephrons and prevented from escaping by the atresia of the pelvis and upper part of the ureter. The significance of the urea concentration in the cyst fluid in this case is not clear, as at the time of sampling the existence of two types of cyst was not suspected.

Congenital stricture of the ureter was observed by Campbell in 0·6 per cent. of autopsies in children. In one-third of these the stricture was at the pelvi-ureteric junction.

Idiopathic dilatation of the urinary tract is a term used to describe cases in which dilatation is present without obstruction. It is assumed to be due to neuro-muscular imbalance, like achalasia of the cardia. Campbell found the condition in 0·9 per cent. of autopsies ; in most cases both ureter and pelvis are affected, and in half the cases the lesion is unilateral.

The non-patent *ductus arteriosus* here reported cannot be the result of anomalous development of the aortic arch system. No other conclusion can be drawn from the descriptions and diagrams in standard works. Had this ductus been derived from the right sixth aortic arch instead of the left, then either it would have emptied into the right subclavian artery or the right dorsal aorta would have persisted in whole or in part ; but no such thing was found. Similarly it seems impossible that the dorsal attachment of a ductus originally right-sided should migrate so that it emptied into a left-sided vessel.

Congdon (1922), in a study of the aortic arch system, describes the development of the pulmonary trunk, primitive pulmonary arteries and ductus arteriosus. Between the 13-mm. and 24-mm. stages the origins of the primitive pulmonary arteries approach one another and also move dorsally, so that the pulmonary trunk increases in length and the ductus arteriosus decreases. In embryos of less than 40 mm. the origins of the pulmonary arteries are in contact. At this stage the ductus is a relatively wide vessel, from which the much narrower pulmonary arteries project like a V. Later stages are not described by Congdon, nor has any such description been found in a number of embryology books consulted.

It is clear that in the processes described by Congdon there occur considerable remodelling of vessels and movement of the attachments of the pulmonary arteries through the substance of the pulmonary trunk. It seems reasonable to suppose that similar processes go on subsequently, which might finally produce the state of affairs normally observed. But preliminary observations suggest that such processes are normally post-natal. It is hoped to make a further study of this question.

It may also be noted that this ductus was more completely closed than is usual at this age.

Regarding the period of intra-uterine life during which the renal lesions developed little can be said, save that it was probably after the 3rd month.

Summary

A case is reported in which the following developmental anomalies were found :—right polycystic kidney, right uretero-pelvic atresia, left hydronephrosis and hydro-ureter without obstruction. This combination of urinary tract congenital lesions has not been previously described, though the individual lesions are well recognised.

There was also a ductus arteriosus springing from the right pulmonary artery. The development of this is not understood, but it is considered not to be the result of an anomaly of the embryonic aortic arches.

Right talipes equino-varus was also present.

We are indebted to Professor W. D. Newcomb and Dr Reginald Lightwood for encouragement and advice, to Dr D. H. Nelson for the radiograph, to Dr L. Crome for help with translation, to Miss B. M. Still for the chemical analyses and to Mr D. Morritt for the sections.

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OBITUARY NOTICES OF DECEASED MEMBERS

Archibald Sutherland Strachan

Born 23rd November 1891. Died 20th February 1949

(PLATE CXII)

ARCHIBALD SUTHERLAND STRACHAN, professor of pathology in the University of The Witwatersrand, died in the Johannesburg General Hospital on the 20th February, 1949, at the age of 59 years. Born in Glasgow on 23rd November 1891, he was the seventh child in a family of 9 sons and 1 daughter. He received his school education at Glasgow High School and entered the University of Glasgow, where he took the degrees of M.A. (Hons.) and B.Sc. in pure science. After this his interest changed to medicine, and a distinguished career as medical student was followed by graduation in 1918. After a short period of military service. until the end of the first world war, he returned to Glasgow to join the staff of the department of pathology under Sir Robert Muir. In 1919 he went to South Africa to the department of pathology and bacteriology of the University of Cape Town.

Two years later, at the request of his old chief, he returned to Glasgow to become senior lecturer in pathology at the University. In 1924 South Africa called him back as a senior pathologist to the South African Institute for Medical Research and senior lecturer in pathology in the University of the Witwatersrand, where he was appointed professor of pathology in 1943. He was awarded the M.D. degree of the University of Glasgow in 1929 and in 1934 he took the M.R.C.P. London.

Strachan's research work was concerned mainly with problems of silicosis, and in conjunction with F. W. Simpson and the workers of the Miners' Phthisis Medical Bureau, Johannesburg, he did much to elucidate the pathogenesis of this disease. It was, however, mainly in the realm of teaching and medical administration that he left his mark on South African medicine. As a teacher he had few equals. Every student became to him a friend whose problems he understood and in whose difficulties he was always prepared to offer valued advice. He occupied the unique position in South Africa of having been intimately concerned with the departments of pathology in each of the three medical schools, almost since their inception. He had been external examiner at the University of Cape Town since 1924 and at the University of Pretoria since 1945 (the end of the first course in pathological anatomy). He had also been associated as external examiner in pathology with the veterinary laboratory at Onderstepoort.

so that there are few if any students who qualified in medicine or in veterinary science in South Africa in recent years who had not passed through his hands. Almost every doctor in South Africa regarded him as a personal friend. In recognition of his services, both as examiner and in many other ways, the University of Pretoria granted him the honorary degree of M.D. in 1948.

Among the official positions he had held were those of Member of the Federal Council of the South African Medical Association, Dean of the Faculty of Medicine of the University of the Witwatersrand, President of the Southern Transvaal Branch of the South African Medical Association, Vice-President of the National Cancer Association of South Africa, and Member of the South African Medical Council since 1943, becoming its Vice-President in 1949. His clear-thinking brain, so adept at clarifying a confused situation or at keeping a meeting in order, will be greatly missed by all these bodies.

Eighteen months before his death, he was informed that he had multiple myeloma in a very advanced stage. None knew better than he the implications of the diagnosis. In spite of this, however, and in spite also of continual pain, he performed his duties, academic and extra-academic, to the full, sparing neither his mind nor his body. Throughout the inexorable progress of the disease and even after suffering a collapse of the 4th cervical vertebra in January of this year, he retained his unbelievable courage, patience and cheerfulness, remaining an inspiring example to all who came in contact with him.

The real love his own students had for him was revealed in a particularly touching manner when, during the last stages of his illness, they took it in turns to be on duty outside his room day and night in case there might be something they could do to be of service to him. By his death South African medicine has lost a unique figure, the doyen of its pathological anatomists, the sage adviser on its councils, and the beloved friend of the whole profession. He is survived by his wife, a daughter and an adopted son.

<div style="text-align: right">

BASIL J. P. BECKER
JAMES BARNETSON

</div>

ffrank William Simson
Born 1st August 1883.   Died 17th December 1948

(PLATE CXIII)

To those who knew him personally the passing of F. W. Simson on 17th December was a tragic blow only slightly softened by the fact that it was not unexpected. But though his death is acutely felt by a comparatively small circle within the profession (for he never set out to be a public figure), it is a loss to every practitioner of medicine in South Africa. He was one of the old school of histopathologists who, by a combination of thorough training, keen powers of observa-

A Sutherland Strachan

tion, a contemplative turn of mind and a rare gift of meticulous care in all he did, gained for himself a well-earned reputation as the most able histopathologist in Southern Africa.

An Australian of Scots parentage, Dr Simson qualified in medicine at Edinburgh in 1918 at a comparatively late age, after an adventurous career as a young man, farming in Australia and trading in the South Sea Islands.

Following graduation he went to Sheffield where, after a term as resident medical officer at the Sheffield Royal Hospital, he joined the staff of Sheffield University. A fundamental thinker and gifted with fine technical ability, he would have been outstanding as a surgeon but, influenced by the advice of Sir Arthur Hall, his interests were turned to pathology and to that specialty he remained faithful for the rest of his life. After eight years in association with such men as Sholto Douglas and Edward Mellanby, he had attained the status of senior lecturer in the department of pathology. He was elected a member of the Pathological Society in 1922.

But routine teaching held no appeal for him and in 1926 he came to South Africa to join the staff of the South African Institute for Medical Research. Within this organisation he remained as its senior pathologist for the next 20 years. There, in addition to his routine work as head of the pathological department, he carried out several lines of research the chief of which were concerned with the ætiology and pathogenesis of the pneumoconioses.

In collaboration with the late Drs Irvine and Strachan a comprehensive picture, radiological, clinical, histological and post-mortem, of silicosis and tuberculo-silicosis as seen on the Rand was gradually built up. These three men, each an outstanding personality in his own sphere, did more than any others to place on a firm basis our knowledge of silicosis as it occurs under the particular conditions pertaining to the gold mines of the Witwatersrand. By his enthusiastic and abounding energy Irvine built up the Miners Phthisis Bureau and made the mode of selection and the care of miners on the Rand second to none. In addition he gradually pieced together the clinical and radiological picture of silicosis. From the available post-mortem material Strachan elucidated the pathological appearances of the disease and correlated them with the radiological and clinical findings. At the same time Simson, with Strachan's assistance, carried out the experimental and histological work which identified the dangerous types of dust on the Reef, demonstrated their effects on the animal body, and elucidated the histopathology of the disease as found in man. Working as a closely co-ordinated team they amassed an astonishing amount of information which was published in several Institute monographs and in various medical journals.

Soon after arriving at the Institute Simson became interested in the problem of asbestosis and carried out investigations on the sputum and lungs of asbestos workers which enabled him to work out the

pathogenesis of this condition. His conclusions, at which he had arrived independently, were anticipated by the publication of Cooke's and McDonald's observations in 1927, and so he published (jointly with Strachan) only a part of the results obtained. (This *Journal*, 1931, xxxiv, 1.)

It is said that genius lies in an infinite capacity for taking pains and if this be true Simson possessed a large share of genius. To see him plan an experiment and then, week by week, month by month, follow it through, checking (and usually performing) every step of the work himself was to see a research worker at his best. In this type of work he excelled, and practically all our knowledge of the pathogenesis and histogenesis of silicosis in South Africa is due to his patient and meticulous work.

Later in life he brought the same thoroughness to the study of various fungal diseases and he did much to elucidate their place in the occupational diseases of South Africa. He was the first to recognise and record a case of histoplasmosis in Africa and to his careful investigation is due our knowledge of the various types of chromo-blastomycosis found in the Union. A unique opportunity of furthering his interest in fungal diseases occurred when an outbreak of over 2000 cases of sporotrichosis in mine workers enabled him to study the histopathology of this disease in man and to work out the life cycle of the fungus both *in vivo* and *in vitro* in the laboratory. Unaware at the time of Gougerot's almost forgotten work on that particular aspect of sporotrichosis, Simson rediscovered the asteroid form of the fungus in the tissues of man and animals. As a result of his work he was able to present a complete histopathological picture of the condition, and, in his valuable contribution to the Transvaal Chamber of Mines publication, to survey every aspect of the outbreak. A feature of this publication, as of all Simson's published work, is the finely detailed photomicrography. Nothing but the best would ever satisfy him and many a technical assistant's heart was nearly broken by his demand for yet more clarity and precision in photomicrographs. In the end, however, the results always justified his insistence, and another article was brilliantly enhanced by its illustrations. It was by means of accurate photomicrography that Simson built up his ingenious model of a bronchus and its related structures which demonstrated beyond all doubt the precise site of the silicotic nodule in the lung architecture. The method he used was described in this *Journal* in 1935. It is a tribute to the thoroughness of his research work that none of his results has ever been challenged.

Simson never enjoyed teaching to large classes and was thoroughly upset by the prospect of reading a paper to an audience, but in the seclusion of the laboratory he excelled in imparting his knowledge to one or two of his juniors at a time. Many a histopathologist will forever remember the teachings of this truly fine man and will be grateful to him.

As in his professional, so in his private life, Simson never sought the public stage of medical politics or social events, but in his circle of intimates he radiated a quiet sense of friendliness and of humour which made him beloved by all who knew him. A scratch golfer, a capable fisherman, a crack shot, an adept at billiards and bridge and a fine figure skater, he exhibited in sport the thoroughness that was the keynote of every aspect of his life. Nothing angered him more than to see slipshod work and none who was willing to give less than his best lasted long as his colleague or assistant.

During the last two years of his life he joined Drs Johnstone and Dew in Durban, and during this time he built up the pathological department at Addington Hospital. To those who knew the serious nature of the illness which first became apparent ten years before his death, his quiet courage never ceased to be a source of amazement and of inspiration.

Though Simson may not have appeared in the public eye as a prominent academic figure, he will live in the memory of those who had the privilege of knowing him intimately as a great teacher, an inspiring personality, an exemplar of thoroughness in all things and a dearly beloved friend.

JAS. F. MURRAY

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William Susman

Born 19th August 1895. Died 23rd December 1948

(Plate CXIV)

William Susman was a Canadian of Latvian-Jewish descent. He was born in Montreal and educated at Queen's University, Kingston, Ontario, where he obtained the degrees of B.A., M.D. and C.M.; he was also a Member of the College of Physicians and Surgeons of Ontario. His interest in pathology declared itself early and he was a student-demonstrator under James Miller in 1922-23. In 1923 he qualified in medicine and was awarded a Hoffmann fellowship for research in pathology. With this he went to Edinburgh, where he spent two years; during the first he studied under Lorrain Smith,

during the second he was one of Smith's assistants and also assistant
pathologist to the Royal Infirmary and pathologist to the Royal
Mental Hospital, Morningside. In 1925 he came to Manchester as
assistant lecturer in bacteriology in the Department of Pathology
under Shaw Dunn and began a lifelong association with the Royal
Infirmary. In 1926 he undertook the diagnostic histology for the
Royal Eye Hospital and continued to do it for the rest of his life.
He was for many years the senior lecturer in the Department of
Pathology, first under Shaw Dunn, then under Baker. At the time
of his death he held the posts of lecturer in morbid anatomy and
histology in the University of Manchester, senior pathologist to the
Manchester Royal Infirmary, honorary pathologist to the Royal Eye
Hospital and honorary pathologist to the Manchester Jewish Hospital.

Susman was a keen research worker and the author of 26 papers,
the last being published posthumously. They cover a wide range of
topics but his dominant interest lay in endocrinology. He had a
sound knowledge of the morbid anatomy and histology of the ductless
glands but his speculative mind tended to carry him into fields in
which morbid anatomy alone is inadequate and where the problems
encountered can only eventually be cleared up by the combined work
of organic chemists and experimental endocrinologists. He was an
experimentalist by instinct but much of his experimental work is
inconclusive and his best papers are those in which he confines himself
mainly to the factual morbid anatomy and histology of the human
subject.

In 1930 he wrote one of the early papers on atrophy of the adrenals
as a cause of Addison's disease. At that time this lesion was accepted
as the cause of about 15 per cent. of cases of the disease. Susman
emphasised that it was a much more frequent cause, particularly in
this country. This was subsequently corroborated by other workers
and in 1936 he was able to collect 28 examples in 65 cases of Addison's
disease reported since 1930. The cause of the atrophy remained (and
remains) obscure.

He was particularly interested in the pituitary and spent much
time on the elusive problem of how to interpret its cytological changes
in terms of functional activity. His best work in this field was in 1935
on the incidence of microscopic adenomas of the anterior lobe. He
found basophil adenomas in 3 per cent. of routine autopsies and
threw doubt on the significance of these tumours as the cause of
Cushing's syndrome. Subsequent work in various parts of the world
has supported his conclusions on this subject.

His early work on the islets of Langerhans led him in 1942 to a
very careful measurement of the amount of islet tissue in various
diseases and to a demonstration that there was a true reduction in
patients with diabetes mellitus. His work on polypi coli confirmed
Stewart's findings that this condition is present in about 5 per cent.
of autopsies ; another example of a " rarity " which turns out to be

PLATE CXIV

common if looked for carefully. He had over 20 years' experience of eye pathology, and the paper he wrote in 1938 on intra-ocular tumours forms the basis for a classification which has found a place in the standard English text-book on the pathology of the eye.

Susman performed or supervised over 10,000 autopsies and collected over 50,000 slides. The great experience represented by these figures was invaluable to his colleagues and to many others who sought his advice. He was a most helpful consultant, always courteous and patient and at pains to explain fully the grounds for his opinions ; for this reason junior colleagues approached him with ease and confidence. He was no less helpful to senior colleagues, who valued in particular his special knowledge of the pathology of the nervous system, the endocrine organs and the eye. His services as a consultant were greatly appreciated and his influence on the work of others has been considerable.

Teaching was one of Susman's main interests and he showed a devotion to it that carried him beyond his formal obligations. His teaching was orthodox and he had no taste for discussing controversial matters. He never lost sight of the fundamentals of any topic, even of those on which he was an expert ; in this he showed a deep insight into the needs and capacities of the average medical student. He was adept at extempore teaching in the post-mortem room and a clear and forceful lecturer. In his practical classes he was strongly opposed to methods reminiscent of the school class-room and believed in allowing students the greatest possible freedom. He made it clear that the Department's function consisted in providing opportunities and that the students themselves were responsible for their training. His policy as a teacher of practical pathology was vindicated by the examination results and won the support of those who assisted him. He was a fair and considerate examiner, always eager to reward special merit or glad to discover extenuating circumstances.

In recent years administrative work, both within and without the University, had claimed much of his time. He was an active member of the Board of the Faculty of Medicine and of its various committees. A member of the British Medical Association since 1924, he served on three of its committees : the Non-Professorial Group Committee (1937-1946), the Special Practices Committee (1938-1939) and the Special Committee on Patents (1944-1945). He was one of the founders of the Non-Professorial Group and the first chairman of its committee, holding this office for about ten years.

Susman was a vigorous and usually a genial personality. though the shortcomings of others sometimes moved him to explosions of wrath that revealed him as a master of terse and picturesque language. A fondness for gadgets and labour-saving devices exposed him to constant twitting, which he bore with good humour, though convinced that his critics were behind the times. He knew the importance of his profession but did not take himself too seriously and his bearing

could hardly be described as academic. Manchester had seen him in his doctor's robes, but these soon made their way back to Canada. He joined our Society in 1926 and was a Fellow of the Royal Society of Medicine and a member of the Manchester Medical and Pathological Societies, but he disliked meetings and reading was his chosen method of keeping up to date.

Susman liked precision and tidiness and was impatient of the doubts and hesitations that punctuate the lives of most pathologists ; he loved a definite diagnosis, and after reading some of the autopsy reports of his more diffident colleagues, declared that most of their patients ought to be up and about. His writing was careful and he had a strong preference for his own forms of expression, even when his friends thought they could be improved on. He was a most acceptable colleague, punctilious in the performance of his routine duties and considerate of his associates ; his last message, sent on the day of his death, clearly showed his concern both for his colleagues and for his work.

Susman saw military service in both wars. When war broke out in 1914 he was a student in arts and a member of his University O.T.C. He tried to enlist in the Canadian Engineers, who considered him too young, but was accepted as a private in the Canadian Army Medical Corps. In 1915 he served in England and Egypt ; in 1916 he was in France, where he was commissioned in the 8th and 2nd Duke of Wellington's West Riding Regiment. Soon afterwards he transferred to the Royal Flying Corps as an observer, who in those days was also navigator, bomb-aimer and rear-gunner. In May 1918 during an air duel he received wounds in both feet that ended his active service and troubled him on and off for the rest of his life. He was demobilised in 1919. In the last war he was a Medical Officer in the Home Guard with the rank of Major and was also an Air Raid Warden.

Susman found delight in a variety of hobbies, especially photography and philately. He read widely among the books of the day but his chief reading was in anthropology. He was a Hebrew scholar and collected a fine library of Hebrew literature, which interested him chiefly from the anthropological standpoint.

Susman's fatal illness lasted less than a day and although there was no autopsy, its nature was obvious. A cerebral hæmorrhage starting during the night presently deprived him of speech, but for a time he was still able to write. The notes he made show that he knew the nature and outcome of his illness and that he bore it with courage. He is survived by his wife, whom he married in 1923, and a son, who is a medical student in the University of Manchester.

We are indebted to the *Manchester University Medical School Gazette* for the portrait of Dr Susman.

S. L. Baker
H. L. Sheehan
Raymond Whitehead

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William Austin Robb

1893–1948

EVERY one of Robb's friends and colleagues would be grateful for an opportunity of paying tribute to him as a colleague who did so much more for them than most men, both professionally and socially.

Born at Burnt Fen, near Ely, in 1893, William Austin Robb came of Suffolk yeoman stock and inherited from them a passion for animals and for the country that never left him and which led him in middle life to give up assured prospects as a consulting physician in London to make his home and career in the surroundings in which he belonged, the countryside of Devon.

Robb commenced his professional life as a pharmacist, taking his major pharmaceutical diploma at the age of 21. Enlisting as a volunteer in the R.A.M.C. in 1914, his efficiency became a byword, the *Pharmaceutical Journal* of 26th July 1919 containing the following passage :—" I would like to compliment the man in charge of the dispensary of the 31st F.A. Had you seen his pharmacy under indescribable conditions you would have wondered how he did it."

This aptitude for orderliness never left him and was still obvious in his later reports, which never required the tidying-up of loose ends. He expected the same high standard from his technicians, so that his laboratory became a first-class training ground for these men, many of whom now occupy high positions in laboratories elsewhere. Severely wounded and captured in 1916, he recovered sufficiently to care for British and German injured in hospital, without discrimination. His subsequent attitude to life was considerably coloured by these grim experiences. He was a man of deep religious feeling but devoid of any aggressive element. He had a keen sense of humour and an innate kindliness combined with sound common sense which enabled him to exude confidence and did much to contribute to his success as a consultant.

Robb's army experience altered his professional outlook and on demobilisation he entered St Bartholomew's Hospital as a medical student, qualifying in 1924 and obtaining the London M.D. and M.R.C.P. in 1926. He was elected F.R.C.P. in 1945. After qualification he served as house physician and chief assistant to the late Sir Walter Langdon-Brown, and appeared to be set on the path of consulting medicine when he surprised all his colleagues by accepting the post of pathologist to the South Devon and Exeter Hospital in 1931. Though not the founder of the pathological department at the hospital, Robb was its first real specialist head. The small building that had been erected for his predecessor (" Solly's Temple ") soon proved to be quite inadequate for an energetic young man. and within a few years of his arrival, in 1935, large extensions were added. These in turn soon became inadequate and in 1947 further additions were made, this time including premises for an assistant pathologist, whose appointment was long overdue.

Robb was probably best known in pathological circles as one of the band of pioneers of clinical pathology—with the accent on clinical —for which his earlier work as a physician had so admirably fitted him. The making of clinical pathology into a consultant service of real value to the doctors (and patients) of Devonshire was done by ceaseless and countless journeys into the countryside for which he was responsible. Indeed he went even further afield, for he held appointments not only at Exeter, but also in parts of Somerset and Dorset, with a radius of some 60 miles around his home town. For this reason Robb wrote but little : there was no time for academic studies, but it must be regretted that his wealth of practical experience has gone unrecorded. On the other hand, a long list of local learned and scientific societies benefited greatly from his experience gained during his long tramps over the countryside. His interest in local affairs was further shown by his membership of the post-graduate sub-committee of the University of the South-West, in whose activities he took a keen interest.

Robb's capabilities are further indicated by the many official

appointments he held. In his early days he was a teacher of pharmacology at Barts., and later he became a member of the revision committee of the B.P. Codex, an appointment he held until his untimely death. He was for some time a member of council of the Association of Clinical Pathologists and during the visit of the British Medical Association to Plymouth in 1938 he was a vice-president of the section of pathology. His was a familiar face at the meetings of the Association of Clinical Pathologists and of the Pathological Society, of which he had been a member since 1935. In both these bodies he will be sadly missed for his boundless energy and friendly humour. He was pathologist to the Exeter and Devon Constabulary and took a keen interest in forensic medicine, on which he became the recognised authority in this part of the county. He maintained a close association with the Exeter city health authorities and gave them every possible assistance in the development of their schemes.

During the second world war Robb was an exceptionally busy man and his laboratory was, of course, greatly understaffed. Indeed it is a source of wonder how he maintained the efficiency of his service over so large an area. But maintain it he undoubtedly did, and his premature death may not be wholly unconnected with the worries of those times. In addition to his ordinary work he undertook duties at all the E.M.S. hospitals in his district, as well as the organisation of the blood transfusion service—a formidable undertaking. When the pre-clinical departments of the Royal Free Hospital Medical School were evacuated to the Washington Singer laboratories in Exeter his was the welcoming hand that met them and the many friends he made during this time became welcome guests in his home, like his own old fellow-students. He died on 12th July 1948.

Robb was exceptionally happy in his home life and every caller was made welcome. He is survived by his wife, and by a son and daughter at school.					F. D. M. HOCKING

Thotakat Bhaskara Menon

### Born 4th May 1898.	Died 12th September 1948

(PLATE CXV)

THE untimely and sudden death of T. Bhaskara Menon just after his arrival in London has robbed India of a brilliant pathologist and medical educationalist. He had been deputed by the Government of India to make a first-hand study of the progress made in medical science in recent years in the United Kingdom and the United States of America.

T. B. Menon was the son of a gifted surgeon and chief medical officer of the Cochin State, who, even in those very early days, went

Plate CXV

to England for higher medical studies. Menon himself, after his collegiate training in Ernakulam College, joined the Madras Medical College in 1916. Qualifying in 1921, he worked for some time in the Cochin Medical Service, which, however, did not appeal to him. He went to England for post-graduate studies and took his M.R.C.P. in 1926. In London he worked as a research student in pathology under Professor Boycott at University College Hospital and Medical School and later under Professor Lorrain Smith in Edinburgh. On his return to India in 1927 he joined the Madras Medical Service and worked as assistant professor of pathology in the Madras Medical College for a period of 4 years. In 1928 he took the M.D. degree of Madras University in pathology, including bacteriology.

Having felt the need for a good text-book on tropical pathology for students, he published in 1931 *An introduction to tropical pathology*. In 1932, he acted as professor of pathology in the Andhra Medical College, Vizagapatam, for six months and was then re-posted to Madras as a lecturer in pathology and bacteriology in Stanley Medical School. Menon's early incursions into the field of research can be passed over briefly. They concern mainly some of the pathological aspects of chronic appendicitis and chronic colitis and he wrote several papers on these and other subjects. During 1933 he undertook a study of granuloma inguinale, its bacteriology and experimental transmission, his final results being published in 1935 in the *Transactions of the Royal Society of Tropical Medicine and Hygiene*. He described the morphology of the Donovan body found in these lesions, compared them with the organism recovered on cultivation and showed that they were morphologically similar. He also produced lesions in the experimental animal by injection of the cultivated organism.

During this period he also studied the much neglected problem of filariasis and was honoured by an invitation from the Madras University to deliver the Maharaja of Travancore Curzon Lectures on " Problems in filariasis " in 1934-35. The filarial problem is one of extreme importance in the tropics, and particularly so in South India. Later, during 1930-41, he carried out a long series of investigations on filariasis, supported by a grant from the Indian Research Fund Association, on the behaviour of the infective larvæ of *Wuchereria bancrofti*, with special reference to their mode of escape and penetration of the skin, the preservation of the microfilariæ *in vivo*, and the mechanism of exsheathing. In 1934 he worked on lizard filariasis.

In 1936 he went again to Edinburgh and worked on splenomegaly under Professor Murray Drennan. In 1937 he was awarded the degree of D.Sc. by Edinburgh University, gaining the Straits Settlements gold medal for the excellence of his thesis in the following year. From Edinburgh he went on a study tour to Vienna and Berlin, spending several months in various pathological institutes in these countries. Menon became a member of the Pathological Society of Great Britain and Ireland in 1938.

On his return from Europe in 1938 he was posted as professor of pathology at Andhra Medical College, Vizagapatam. It was after his return that he directed the research on filariasis already mentioned, work which received due recognition. He was elected to the Fellowship of the Royal College of Physicians of London in 1940, an honour which gave him the greatest satisfaction and increased his sphere of activity and influence. In 1945, the Government of Madras, in consideration of his merits, appointed him the first Indian permanent principal of the Andhra Medical College. He disliked administrative work, but he had to accept it, and consequently his scientific work, which he loved so much, suffered. However, from 1946 he had been directing research on the subject of cirrhosis in animals and its bearing on the ætiology of infantile biliary cirrhosis. This enquiry is not yet completed, but the work done so far is in course of publication.

The Army Command of India requisitioned his services as professor of pathology for the newly created Army Medical College at Poona and consultant pathologist for the Army with the rank of colonel. He accepted the job and was to have joined in November on his return from abroad. He had also made arrangements to take his trained staff to Poona to continue the work on cirrhosis, but, alas! fate snatched him away at a time when his country needed him most. India has lost an experienced research worker who would have played a prominent part in the reorganisation of medical research in his own country and it will be very difficult to fill the void.

In his comparatively short life, Menon had contributed extensively to the literature of pathology, embodying therein original observations on many subjects. He was a delightful professor, treating his assistants and students as his equals and laying before them without stint the riches of his mind. And indeed he had a well-trained and disciplined mind, and his freshness of outlook and his enthusiasm were both exhilarating and infectious. By his talks at the tea-table about institutions and personalities in Vienna, Edinburgh, London and elsewhere he used to enthuse his juniors and so help to foster a scientific atmosphere. His lectures were truly a model of teaching —lucid, precise and unambiguous, with the right emphasis on essentials.

His sudden death at the early age of 50 came as a great shock to all his friends, colleagues and students and it has deprived India of a brilliant pathologist, research worker and educationist. He leaves an only son and an aged mother, to whom we extend our deepest sympathy.

I. BHOOSHANA RAO

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Observations on the pathology of hydrocephalus

By DOROTHY S. RUSSELL. Medical Research Council Special Report Series no. 265. 1949. London : H.M. Stationery Office. Pp. vi and 138 ; 90 text figs. 6s.

Since the experimental work of Weed and of Dandy the causation of hydrocephalus has been too often interpreted in too facile a fashion. The cerebro-spinal fluid is formed by the choroid plexuses, traverses the ventricular system and is eventually mainly absorbed into the venous sinuses by way of the arachnoid granulations. With such a clear-cut physiological process it is obvious that hydrocephalus usually results from obstruction to these pathways. Yet apart from cases of hydrocephalus produced by tumours in the central nervous system, the morbid anatomy of this disease process has not always been clearly demonstrated, " It is not necessary to study the different works on hydrocephalus very exhaustively to find that actually observed lesions are much rarer than theories explanatory of the causes of hydrocephalus." This defect Professor Dorothy Russell's monograph goes far to remedy.

The classification of causation under maldevelopment, inflammation and neoplasm follows the usual course, but, unlike most pathologists, Professor Russell has succeeded in avoiding the term idiopathic. Careful anatomical and histological investigation has succeeded in demonstrating obstruction in all cases. She has, more clearly than has been done hitherto, demonstrated and discussed the various stenoses of the aqueduct—those due to maldevelopment and those due to acquired gliosis. Of particular interest is the discussion on " forking " of the aqueduct and the demonstration of its congenital nature. The Arnold-Chiari malformation of the hind brain is clearly defined and the conclusion reached that traction of the spinal cord has no part in its formation. The fact that decreasing grades of this deformity accompany the less severe manifestations of spina bifida does, however, suggest some relationship between these two lesions.

In these days of chemotherapy and antibiotics this monograph should serve a useful purpose. The necessity for early and optimal treatment of inflammation if meningeal adhesions and obstruction are not to complicate the eradication of infection is stressed. There is a critical discussion of the so-called " otitic hydrocephalus," but the pathology of this condition is still undemonstrated.

The great merits of this monograph are that it assembles in compact form all that is really known about the subject, analyses critically a large series of cases which have been well studied both clinically and pathologically, and finally brings home to the pathologist that the conclusion that any case is idiopathic is an admission of imperfect study or technique. Professor Russell is to be congratulated upon the production of such a sound piece of critical work.

Morgagni's syndrome : Hyperostosis frontalis interna, Virilismus, Obesitas

By FOLKE HENSCHEN. 1949. Edinburgh : Oliver and Boyd. Pp. xii and 172 ; frontispiece, 99 figs. on 13 plates and 13 text figs. 30s.

Morgagni, in 1761, described the gross appearances of hyperostosis frontalis interna in the skull of a 75-year-old woman " virili aspectu et valde obesa." It was this which led Professor Henschen in 1936 to name the syndrome after him and he has brought forward strong evidence for virilism and obesity being the most constant associates of hyperostosis frontalis interna. The full triad was present in 47·4 per cent. of 266 cases presenting hyperostosis at autopsy, and either obesity or virilism existed in a further 40·6 per cent. " Morgagni's syndrome," says Henschen " is on the whole only slightly pathological and, in fact, need not be accompanied by any clinical symptom at all." He does not consider it justifiable to separate off a special *Stewart-Morel syndrome of hyperostosis frontalis* interna, adiposity and cerebral disturbances, since the latter may well be the result of the fortuitous occurrence of senile cerebral and vascular degenerations.

The endocrine background of Morgagni's syndrome is still imperfectly understood. It is discussed in connection with the skull changes known to occur in pregnancy, in acromegaly and in Cushing's syndrome. Histological abnormalities in the pituitary, principally a decrease in the chromophobe cells, have been observed, but such qualitative surveys are not dependable. No gross cortical hyperplasia or adenoma of the adrenal glands was seen in any case with " virilisme pilaire." Hyperostosis frontalis interna is about 100 times more common in females than in males. Much of the latter part of the book is occupied by a discussion of points such as these.

The earlier chapters are devoted to a scholarly and comprehensive review of the relevant literature over the past 200 years, and to individual case records selected from the author's own material. There is also a fully illustrated section on the naked-eye, microscopical and radiological features of the cranial hyperostoses.

It is not possible to do more than indicate the scope of the book. Many other circumstances surrounding the occurrence of hyperostosis frontalis interna are discussed. The book is not easy to read ; there is too much academic detachment in its writing, so that its main argument and conclusions tend to be hedged around with limitations conscientiously observed. Certain passages would carry more conviction if formal statistical analyses of the data had been presented, together with the tables and charts. Professor Henschen is scrupulous in not giving the impression that his is the last word on the subject. It is, however, the latest word, and this book will provide a completely satisfactory *point de départ* for any future studies. As such, it must find a place in all medical libraries. It is essential to those interested in the subject. Those not so interested may profitably consult it. Then let them return to the post-mortem room and see if they can, with Professor Henschen, identify at least some degree of hyperostosis frontalis interna in 40 per cent. of the calvaria they remove from post-menopausal women.

The production of the book is, of course, excellent, its format being closely modelled on this *Journal.*

Racial variations in immunity to syphilis

By CHESTER NORTH FRAZIER and LI HUNG-CHIUNG. 1949. Chicago; University of Chicago Press: London; Cambridge University Press. Pp. xi and 122 ; 13 text figs. 14s.

In this very interesting study a large series of cases of syphilis occurring in the Chinese in Peiping is compared with a similar series compiled in an American hospital and including both whites and negroes. The figures are sufficiently large for the observations to be significant. The authors point out that the disease is essentially the same in all races. Differences between races lie almost entirely in the relative frequency with which the various phenomena of the disease develop.

Among other remarkable observations are the fact that general paralysis was six times more frequent in the white males than in Chinese males, that the very high incidence of early syphilitic meningitis in the Chinese could be attributed almost entirely to inadequate early treatment, and that, generally speaking, the disease was milder in the Chinese than in other races. In this connection the authors are inclined to stress the well-known androgyny of the Chinese race as a contributory factor. It has been known for a long time that syphilis runs a much more benign course in the female, and in the Chinese there are noted certain qualities of bodily form and mental aptitude which would appear to indicate a strong feminine component in the bi-sexual amalgam of the race.

This study is of great importance in destroying many popular misconceptions with regard to the incidence and behaviour of syphilis in the Chinese and emphasises once again the value of Osler's introduction of the card index system into medicine.

Hematology

By CYRUS C. STURGIS. 1948. Oxford : Blackwell Scientific Publications Ltd. Pp. xii and 915 ; 9 colour plates and 79 text figs. 63s.

The chief virtues of this excellent book are that it is well written, informative, enjoyable to read despite its large size, and different from almost every other hæmatological textbook in its approach to the subject. Dr Sturgis is Professor of Medicine in the University of Michigan and writes as a physician and not as a laboratory man. He therefore never overlooks the fact that blood diseases began by patients having them and that they did not originate in the fertile minds of microscopists. This is not to say that he ignores or belittles the contributions of pathologists and technicians ; indeed every important advance in the scientific study of blood disorders is acknowledged, described, thoughtfully assessed and fitted into the full picture. But technical procedures are scarcely mentioned and none is described in detail, nor is there any pother about the classification and inter-relationship of the various types of blood cells. In the chapter on blood transfusion, for example, no more than an outline description is given of the blood groups and this takes second place to indications for transfusion. The sections on each of the main blood diseases appear under the conventional sub-headings of definition, history, ætiology, symptoms and signs, pathology, laboratory findings, course and prognosis and treatment, with such variations as are appropriate to the particular topic. These sections are knit together by excellently conducted discussions on the broad physiological or pathological aspects of groups of disorders, *e.g.* iron-deficiency anæmias or hæmorrhagic states.

The prodigious toil of writing over 800 pages of text and consulting some 1800 references must have been mitigated for Dr Sturgis by his

evident enthusiasm for his subject and the satisfaction of knowing that he was really writing a book and not merely compiling a catalogue. He has handled his vast material in masterly fashion. He has selected his references both widely and wisely and, in passing, it may be said that he has done ample justice to the work of British hæmatologists of modern as well as ancient days. In his preface he writes " . . . a true and profound insight into any complex subject can only be based upon a historical study dealing with the evolution of each forward step." Accordingly he has taken great pains in preparing long historical essays to introduce almost every chapter. These are written with clarity and judgment from a fount of scholarship, and many readers will, with the reviewer, be deeply grateful to Dr Sturgis for his efforts in this direction. Five pages are devoted to the history of the use of iron in chlorosis and fifteen pages cover the story of pernicious anæmia from Addison to folic acid (B_{12} came too late for inclusion, since the references only reach the year 1946).

The reviewer first put this book to the test as a bench-side companion. but found two failings in it as such. One, the paucity of technical detail, has already been mentioned. The other was that whenever he opened it he became so absorbed that current work was too long interrupted. Only reflective armchair reading was able to do the book justice. This is a book for the post-graduate and particularly for the specialist, whether in the laboratory or in the ward, and for the teacher. They, as connoisseurs, will appreciate it fully and, overlooking its occasional lapses, will be able to add to it their own knowledge of the most recent advances.

The text figures are mostly charts and diagrams with a few photomicrographs. The coloured plates are of representative blood films and are, alas! executed in the usual poster-artist style. One feels that they hardly belong to this book and that they were commissioned by the publishers to decorate what at first glance looks like too much solid text. Enough has been said to indicate the scope of the book and the very favourable impression it has made. One severe criticism must now be entered, but only one. There are far too many typographical and spelling errors—primative, lymptatic, polycytic kidney, Vaughn, proteif and, *pace* the Declaration of Independence, capsul repeatedly for capsule. Even Thomas Addison is twice in the text credited with having written about the " Suprarenal Capsuls," although the missing " e " s are restored in the bibliography. References are given in footnotes, which perhaps spoil the look of the pages but prove to be a great convenience to the reader. They are also collected together at the end of the volume where they are arranged under authors alphabetically.

Diagnosis of viral and rickettsial infections

Edited by FRANK L. HORSFALL, Jr. 1949. New York; Columbia University Press: London; Geoffrey Cumberlege. Pp. x and 153; 7 text figs. and 4 figs. on 2 plates. 21s.

This small volume is the first symposium of the recently organised Section of Microbiology of the New York Academy of Medicine. It covers much the same ground as " Diagnostic procedures for virus and rickettsial diseases " published by the American Public Health Association (see this *Journal*, 1948, lx, 524) but without details of laboratory techniques. In five of the thirteen chapters—those on influenza, the psittacosis-lymphogranuloma group, herpes simplex infections, rabies and rickettsial infections —the authors contributed to the same subjects in " Diagnostic procedures " and there is a good deal of overlap in the two books. It is true that the present volume is directed to the practising physician but much of the

discussion and interpretation of laboratory results would seem to require greater familiarity with laboratory techniques than the average doctor possesses. There are short but good chapters on infectious mononucleosis and infectious hepatitis, subjects which were not dealt with in the earlier work ; but it seems odd to find a chapter devoted to dengue when no space has been found for smallpox.

The significance of some of the tables giving results of laboratory findings was probably more readily understood when the material was presented in lectures than in the printed form, where a fuller explanation would have been desirable ; and while abbreviations such as E.M.H. and P.V.M. may be intelligible to virus workers, it is doubtful if they convey anything to the average clinician. In one respect the book should be valuable both to the clinician and to the laboratory worker ; throughout, the value of the evidence obtained by laboratory tests is critically assessed and the time-consuming and laborious nature of the investigations frequently necessary has been emphasised. The principles of the laboratory methods for diagnosis are briefly stated by the editor in the introductory chapter, and valuable advice is given, in the chapter on typhus by Smadel, on the general procedure to be followed in the investigation of virus diseases ; if these were appreciated by clinicians, the work of the virus diagnostic laboratory would be greatly facilitated and the results of greater assistance to the practising physician.

Bacterial and virus diseases

By H. J. PARISH. 1948. Edinburgh : E. & S. Livingstone. Pp. 168 ; 27 figs. (14 in colour) on 13 plates and 5 text figs. 7s. 6d.

The reviewer cannot do better than quote, as the purpose of this book, the opening sentence of the preface : " This little book has been written to present in convenient form the essential principles of immunology and their practical application in human medicine."

This objective is reached without waste of words and without the omission of any point of importance. A lucid and critical account is given of the place of vaccines and sera in medicine without overstatement of any therapeutic claim. Precise regimes are laid down and authoritative instructions given on details of procedure and on the management of complications such as serum sickness. Diagnostic reagents are discussed with equal precision and include two colour plates of the variants of the Schick reaction. Advances in chemotherapy have lessened the interest in immunology and most of its unsound practices have fortunately died out, such as the therapeutic use of vaccines. The Pasteur treatment of rabies perhaps stands between this use and the true and undisputed prophylactic use. The absence of any reference to the present scepticism as to the value of this treatment of rabies is a slight defect in the book.

This small volume is sound, complete, readable and convenient in form and is to be warmly recommended to both senior student and practitioner.

The filterable viruses. (Supplement no. 2 of Bergey's *Manual of Determinative bacteriology*, edition vi, 1948, with revised and enlarged index)

By FRANCIS O. HOLMES. 1948. London : Baillière, Tindall & Cox. Pp. xxiii and 160. 20s.

This is a magnificent edifice ; we have, however, an uneasy feeling that it has been built too soon and that its foundations are unsound. Or are there any foundations ? Dr Holmes has attempted a tremendous

task—nothing less than the classification and nomenclature of the bacterial, plant and animal viruses, and it is with the plant viruses that we are chiefly concerned in this review. At first sight the system has the look of a serious scientific classification, but what is it based on ? Surely mainly on that most elusive characteristic, disease symptoms, and this leads to a number of anomalies.

The first and most obvious criticism is of the family Marmoraceæ, the mosaic viruses ; this contains six genera of which the largest is the genus *Marmor* ; it occupies 39 pages and contains 67 viruses. In this genus are placed viruses, separated it is true into groups, which have no apparent relationships at all. For example, what are the reasons for grouping dahlia mosaic virus with the tobacco necrosis viruses ? The first is an aphis-borne mosaic virus, not mechanically transmissible, while the second are crystallisable soil-borne viruses lacking insect vectors and easily transmitted by sap. The tobacco necrosis viruses do not even produce a mosaic mottle. On the other hand, potato viruses A and Y, though serologically related, are placed in different groups of the genus *Marmor*. In the family Annulaceæ are grouped the ringspot viruses in a single gene *Annulus*, of which one characteristic is that the vectors are unknown. Yet a virus, recently described, which affects *Tropæolum*, tobacco, and other plants, is a typical ringspot virus with an aphid vector. Should this be included in the Annulaceæ, among the ringspot viruses without vectors, or in the Marmoraceæ among the aphis-transmitted mosaic viruses, since on some plants it produces a typical mosaic and on others a typical ringspot pattern ?

These examples are quoted merely to illustrate the pitfalls of a system of classification based on symptomatology. Dr Holmes has shown immense care and patience in accumulating a vast amount of useful data and great ingenuity in coining the names of viruses, but it seems to the reviewer that the time is not yet ripe for designing a permanent system of classification of viruses. This must surely wait upon the elucidation of the fundamental physical and chemical properties of the viruses themselves, aided by knowledge of relationships based on serological and cross-immunity studies.

These remarks apply with even greater force to the viruses affecting insects, about which practically nothing is known and which are only now being intensively investigated in Canada, California and Cambridge. Preliminary investigations suggest that the number of viruses affecting insects is much greater than was originally supposed and at Cambridge alone some 6 or 7 apparently new viruses have been discovered. We do not yet know whether these viruses are all distinct or nearly related, and it seems premature to say the least to attempt to name and classify viruses about which we know so little.

Diseases of the Chest

By ROBERT COOPE. 2nd ed., 1948. Edinburgh : E. & S. Livingstone Ltd. Pp. xv and 541 ; 42 figs. (8 in colour) on 21 plates and 126 text figs. 25s.

The second edition of this book differs little from the first, though it is 16 pages longer. The increase is mainly taken up by a fuller description with more diagrams of the segmental distribution of individual bronchi and the radiological appearances of their various diseases. The treatment of the pneumonias is brought up to date. Though a few minor criticisms are made, they are not intended to obscure the fact that this is one of the best and clearest books ever written for medical students and practitioners ; authors of more ponderous works must envy Dr Robert Coope's ease and clarity of style and of expression.

Brock's nomenclature of the broncho-pulmonary segments might have been preferred with advantage : streptomycin is not mentioned : in the treatment of rib fractures the use of local anæsthetics is omitted. Decortication and the important principles which underlie its use in hæmothorax and empyæma should be more fully described. The recognition of malignant cells in the sputum is of little help in the early diagnosis of bronchial carcinoma and requires a degree of pathological specialisation and experience only too rarely available. Not enough emphasis is laid on the fact that history, radiology and thoracotomy together form the only sure method of diagnosing early bronchial carcinoma, or that the operability of a case can only be decided after the chest has been opened. The important differential diagnosis between " idiopathic " pleural effusion and the sterile non-purulent effusions which so commonly follow pneumonia treated by sulphonamides should be discussed in greater detail. A short description of the value and technique of laryngeal swabs would have been helpful. These, however, are minor criticisms which will perhaps be of help in the preparation of future editions of this excellent book.

Post-mortem appearances

By JOAN M. ROSS. 5th ed., 1948. London, New York, Toronto : Oxford University Press (Geoffrey Cumberlege). Pp. ix and 308. 8s. 6d.

The fifth edition of this useful handbook appeared in 1948 nine years after the first appearance of the previous edition. As Dr Ross states in her preface its use has spread among a far wider range of workers than those for whom it was originally intended, namely students in the post-mortem room and in the museum. Revision has been effected to meet the needs of this new public, with the addition only of some 30 new pages. Short introductory paragraphs have been written for many of the sections and recent new knowledge about erythroblastosis fœtalis has been added. The appendices which summarise the anatomical normals of the adult and all those irritating details about fœtal length, weight and dates of ossification of principal bones are still there. It is no wonder that the volume was a passport to Dr Ross during the period of her war service. It must surely have been the standby of many a pathologist working in isolated units in time of war. We anticipate future editions and an ever-widening public.

The examination of waters and water supplies

The 6th ed. of Thresh, Beale and Suckling, revised by E. W. TAYLOR. 1949. London : J. & A. Churchill Ltd. Pp. xii and 819 ; 1 plate and 55 text figs. 70s.

The sixth edition of this well-known manual marks a turning-point in its authorship. During more than forty years—the first edition was published in 1904—Dr Thresh and his collaborators have developed the work to its present position, one of general recognition as a standard treatise on water and water supplies, providing information required by sanitary engineers, public health officers, analysts, bacteriologists and others whose responsibilities or interests are concerned with any aspect of the subject. Now the authorship passes to Dr E. W. Taylor and, although the general plan of the book is maintained, there has been a considerable amount of beneficial revision. Obviously the whole book has been thoroughly scrutinised in the process, resulting in an improved balance of presentation. There is evidence, too, of an informed individual critical judgment and

assessment which is welcome. The number of references has been substantially increased. The figures have been rearranged and some new ones added. This edition should enhance the book's reputation and augurs well for its future.

Textbook of bacteriology

By C. H. BROWNING and T. J. MACKIE. Eleventh edition of Muir and Ritchie's " Manual ", 1949. London, New York, Toronto : Oxford University Press (Geoffrey Cumberlege). Pp. x and 907 ; 226 text figs. 50s.

Ten editions of Muir and Ritchie's handbook appeared between 1897 and 1937 and to many generations of medical students this solid compilation was virtually indispensable. Since 1937 there have been great advances in bacteriology and the eleventh edition has needed extensive re-writing. The opportunity has also been taken to have it re-set in a larger format so that the bulky handbook is now an elegant text-book. As in previous editions the pathogenic fungi and protozoa are described as well as the bacteria and viruses which cause disease in man. Antibiotics, the electron microscope and the newer knowledge of chemotherapy are among the many advances which receive due attention. The authors have wisely retained the section on laboratory methods but this now appears in an appendix. In the text the familiar bacterial nomenclature of previous editions is still used but in the chapter headings the generic and specific names adopted in Bergey's Manual are also given. A useful bibliography at the end of the book lists original references for each subject and introduces the reader to reviews which merit further study.

The task of sifting the mass of current bacteriological literature and maintaining a judicious balance between old and new is not easy but the authors have done this in a manner which reflects their great knowledge and experience. Many of the chapters on individual bacteria are excellent but sometimes, as in the description of the staphylococci, the treatment is a little diffuse and the reader has difficulty in getting the subject in sharp focus. The section on viruses gives essential information succinctly but compression sometimes creates impressions which are not intended. Thus the text might lead the reader to believe that the elementary bodies of herpes zoster can be cultured on the chorio-allantois of the developing chick, whereas this has only been achieved on pieces of human skin which have been grafted on egg membranes. As is almost inevitable in such a book there are occasional statements which are not in line with modern views. Paul's rabbit test, for example, is recommended for the diagnosis of variola, although this method has been abandoned by most workers. The retention of the chapter on pathogenic fungi by Dr Cranston Low and the section on pathogenic protozoa will be welcomed by those who need a brief, reliable and easily accessible source of information on the main features of these organisms. While it cannot be said that the book is easy to read, few British text-books cover so wide a field so well. On the other hand it may well prove too comprehensive for the needs of the medical student. This edition will maintain and indeed enhance the reputation so long held by " Muir and Ritchie " as an authoritative account of modern knowledge about micro-organisms.

PROCEEDINGS OF THE PATHOLOGICAL SOCIETY OF GREAT BRITAIN AND IRELAND

8th and 9th July 1949

The seventy-eighth meeting of the Society was held in the University of Oxford on Friday and Saturday, 8th and 9th July 1949

Communications and demonstrations

The communications marked with an asterisk are abstracted below

B. E. HEARD. Irreducible folds in the internal elastic lamina of renal arteries.

A. R. CURRIE. The healing of squamous epitheliomata.

A. C. LENDRUM. Further observations on pulmonary hæmosiderosis.

*M. STRAUB and A. SCHABERG. Changes in the liver in extremely cachectic Indonesians.

J. M. NAFTALIN and J. W. HOWIE. Liver changes in so-called iron-deficiency anæmia of pigs.

*E. LETTERER. Some new aspects of experimental amyloidosis.

R. D. STUART and I. MACINTYRE. Canine leptospirosis.

G. L. MONTGOMERY and I. MACINTYRE. Renal lesions in *L. canicola* infection in dogs.

R. F. OGILVIE. Further observations on the effect of anterior pituitary extract in alloxan diabetes.

B. LENNOX and J. S. PRICHARD. The association of bronchial carcinoma and peripheral neuritis.

J. C. WHITE. Proteins and nucleic acids in the marrow cells.

D. M. PRYCE. A post-mortem technique for slicing the brain *in situ*.

F. A. DENZ. Midzonal liver necrosis in beryllium poisoning.

H. A. SISSONS. Bone changes in experimental beryllium poisoning.

L. DMOCHOWSKI. Some data on the distribution of the milk factor.

L. DMOCHOWSKI and J. W. ORR. Absence of the milk factor in chemically induced mammary tumours of mice.

R. D. PASSEY, L. DMOCHOWSKI, W. T. ASTBURY and R. REED. Preliminary electron microscope investigations of some human material.

C. H. STUART-HARRIS. Death from influenza : a statistical and laboratory investigation.

J. V. DACIE, E. DRESNER, D. L. MOLLIN and J. C. WHITE. Treatment of leukæmia with aminopterin.

ISOBEL HINDE. Glycogen in collecting tubules of young animals.

R. I. K. ELLIOTT and A. H. T. ROBB-SMITH. Hormonal induction of fibromata in scorbutic guinea-pigs.

S. D. ELEK and E. LEVY. Differentiation of staphylococcal hæmolysins by a plate technique.

I. LOMINSKI. Protection of rabbits against Staphylococcus infection by the coagulase-inhibiting factor of serum.

P. HARTLEY. Further observations on the properties of different types of diphtheria antitoxin.

492

A. H. EMSLIE-SMITH. The influence of inoculation on naturally occurring agglutinins in rabbit sera.

A. G. HEPPLESTON. Quantitative airborne tuberculosis in the rabbit : the course of primary infection with human bacilli.

R. KNOX and G. T. COOK. Fermentative variation in Shigella and coliform groups.

A. MACDONALD and A. W. DOWNIE. Inhibition of complement-fixation technique in the study of the pox group of viruses.

C. L. OAKLEY, G. HARRIET WARRACK and IRENE BATTY. Local production of antitoxin.

C. L. OAKLEY and A. J. FULTHORPE. Infinite flocculating systems as indicators in toxin-antitoxin reactions.

C. RICKETTS, J. R. SQUIRES and E. TOPLEY. Skin lipids with special reference to their bactericidal action.

R. A. SHOOTER. The response of staphylococcal skin infections to penicillin.

A. H. E. MARSHALL and R. G. WHITE. Tissue reactions to antigens.

I. MACINTYRE, G. L. MONTGOMERY and R. D. STUART. *L. canicola* infection in dogs.

T. F. HEWER and H. HELLER. Letterer-Siwe disease with diabetes insipidus.

G. T. COOK and R. KNOX. Fermentative variants of Shigella.

G. T. COOK and E. H. CUTHBERT. Pigment variation in a group B Streptococcus.

O. C. LLOYD. The relationship between Lindau's hæmangioblastoma and the angioblastic meningioma.

L. L. R. WHITE. A malignant tumour of the thymus with myasthenia gravis.

J. C. VALENTINE. A cylindromatous type of adenoma of the trachea and bronchus.

W. E. VAN HEYNINGEN. (1) " Monod " culture tubes for measuring growth rates of bacteria. (2) A rapid laboratory evaporator.

T. Y. TAI and W. E. VAN HEYNINGEN. A modified roll-tube method for determining viable counts of bacteria.

K. I. JOHNSTONE. (1) A device for the preparation of glass micro-needles, using a standard microscope. (2) The cultivation of clostridia from single vegetative cells or from spores.

A. G. SANDERS and H. W. FLOREY. Some modified types of Sandison-Clark chambers adapted for the study of the effects of bacteria and antibiotics in the tissues of the living animal.

G. HARRIET WARRACK and C. L. OAKLEY. Tests for hyaluronidases and antihyaluronidases.

H. S. BURTON, G. NEWTON and E. P. ABRAHAM. Purification of antibiotics. Methods of chromatography, and the 54-tube counter-current distribution apparatus on view.

R. M. M. JORDAN. Some aspects of metabolism and growth of *Pasteurella septica*.

A. M. HOLMES and G. P. GLADSTONE. The uses of cellophane sacs in bacterial cultures.

T. F. HEWER. Six cases of adamantinoma of the jaw.

R. A. SLADDEN. Gummatous myocarditis.

J. E. FRENCH. Neurofibromatosis of the appendix.

Abstracts

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HEPATIC CHANGES IN EXTREMELY CACHECTIC INDONESIANS

M. Straub and A. Schaberg

Rotterdam

Shortly after the capitulation of the Japanese in Java we had the opportunity of making a number of post-mortems on extremely cachectic Indonesians. Acute tuberculosis and amœbic and bacillary dysentery were frequently encountered, but in 60 cases hunger cachexia was the essential cause of death. The mean age of the patients was 30 years. Both wet and dry types of hunger cachexia were met with. The mean bodyweight of the patients, taking œdematous and non-œdematous cases together, was 29 kg., the normal body-weight of Indonesians being 40-50 kg.

The liver was as a rule severely atrophic, the mean weight being 871 g. The observed changes in the liver consisted of atrophy, fatty degeneration, hepatitis, cirrhosis and acute yellow atrophy. Often two or more of these conditions coexisted, but always one predominated. Table I gives a summary of our findings.

TABLE I   .

The liver in extreme cachexia (Batavia, 1946)

	No. of cases	Mean weight (g.)	Lowest and highest weight (g.)
Pure atrophy	27	790	460-1067
Fatty degeneration . . .	11	921	480-1402
Hepatitis	10	898	494-1495
Cirrhosis	3	1206	970-1490
Acute yellow atrophy . . .	7	794	484-1453
Atrophy with induration . .	2	1390	1370-1410

Pure atrophy could be diagnosed only by the weight of the liver and microscopically. Two cases that showed typical atrophy microscopically had nevertheless a normal liver weight. This could be ascribed to the general increase in weight of the reticulin network, the fibres of which had undergone a change into collagen. The same was found in lesser degree in most of the atrophic livers. This change should be called induration of the liver tissue and should not be confused with cirrhosis.

Another complication that increased the weight of these purely atrophic livers was the occurrence of larger and smaller nodules of regenerating liver tissue. In one case these nodules could be seen macroscopically ; in other cases they were found microscopically in the centres of the lobules or near the portal tracts. These nodules are pathologically identical with those found in cirrhotic livers or in hepatitis. In the cases mentioned they were a complication of pure atrophy.

Although in 5 atrophic livers there was histologically some fatty degeneration, this remained completely in the background. In 11 cases fatty degeneration dominated the picture, causing a rise in mean weight to 921 g. Normal weight was reached when complete fatty degeneration took place. In other cases fat was only found in the periphery of the lobules.

In 10 cases the changes in the liver could be characterised as general hepatitis. There existed acute and subacute inflammation in the portal tracts, with involvement of the neighbouring liver tissue. There was great polymorphism of the liver cells, dissociation of hepatic cords and often of bile canaliculi. Now and then a nodule of regenerating liver tissue could be found. Cirrhotic changes were lacking. That atrophy was present was shown by the mear weight of 898 g. Inflammation and fatty degeneration were responsible for the larger mean weight and for the wide extremes in liver weight.

In 3 cases annular cirrhosis was noted, the liver showing fatty degeneration with distinct periportal inflammation.

The most interesting group is that of 7 cases of acute degenerative conditions. Icterus was not the predominating clinical characteristic ; the patients died of hepato-renal insufficiency before icterus was clearly established. The kidneys showed the features of Lucké's lower nephron nephrosis. At post-mortem, the diagnosis could be made from the extreme softness of the very small liver. Taking into account the fatty degeneration and inflammatory changes which are often present, the atrophy can really be called extreme. There were two cases of recurring acute atrophy, the left half of the liver showing an older hepatitic stage.

It is of some value to compare these findings with those in non-cachectic patients from the same environment. The most frequent causes of death amongst the latter were war accidents and acute pneumonia. Table II gives the comparison.

TABLE II

Changes in the liver in cachectic and non-cachectic Indonesians (Batavia 1946)

	No. of patients	
	60 cachectic	20 non-cachectic
Mean body weight	29 kg.	39·7 kg.
Mean liver weight	871 g.	1375·0 g.
Mean vitamin-A content of liver . .	39 I.U.	22·2 I.U.
Pure atrophy	27 (+2)	0
Fatty degeneration	11	1
Hepatitis	10	2
Cirrhosis	3	2
Acute atrophy	7	0

The small vitamin-A content of the control cases shows clearly that in these patients nutrition, though not showing in body weight and liver weight, must still have been deficient. Infectious hepatitis was found in 2 cases ; in another case annular cirrhosis was present ; in a fourth, the liver showed portal cirrhosis of Laennec in a distinctly hepatitic stage. Here the nodular hyperplasia dominated the picture. The hepatitis had clearly taken a chronic course. The acute degenerative complications of hepatitis were lacking in this group.

The experimental investigations of Himsworth and Glynn (Glynn and Himsworth, 1944 ; Himsworth and Glynn, 1944-45 ; Himsworth, 1947) have clearly shown that, in rats, atrophy, acute liver necrosis, fatty degeneration and cirrhotic changes can be caused by different types of deficient nutrition. Observations on hunger cachexia in man have not given completely comparable results. This can be readily understood. Hunger cachexia in man is always a complex of several deficiencies. In the winter of 1944-45 in Holland we saw

purely atrophic livers weighing from 700 to 900 g. Uehlinger (1947, 1948) and Lamy *et al.* (1948) found a normal liver weight and only slight microscopical changes in patients coming back from German camps. Hepatitis as found in our material from Java was not a general feature of hunger cachexia in Europe.

Infectious hepatitis and the concurrent acute degenerative liver conditions showed increased frequency in Europe after the first world war and during and after the second. A severe epidemic of infectious hepatitis with a low death rate was seen in the Mediterranean theatre of war. A malign acute course of the disease was found in Burma. Our observations seem to provide another example of the same.

Cirrhosis of the liver is a much more frequent disease outside than inside Europe. Although it can generally be characterised as the portal cirrhosis of Laennec, tropical cirrhosis is much more often encountered in the hepatitic stage. Our observations illustrate the frequency of hepatitis under bad living conditions and with transition to portal cirrhosis when these adverse conditions do not reach an extreme degree.

It may be suggested that chronic malnutrition leads to tropical and sub-tropical populations becoming a reservoir of the virus of infectious hepatitis.

What we saw in extremely cachectic Indonesians differs only in degree from what in Indonesia is considered "normal" features of pathology. Our observations add support to the conclusion of Snell (1947) that cirrhosis is a disease with a nutritional background, if only in the sense that dietetic factors render the liver vulnerable to hepatotoxic substances and to virus infection.

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SOME NEW ASPECTS OF EXPERIMENTAL AMYLOIDOSIS

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Since the days of Virchow amyloidosis has interested a large number of workers, whose investigations have in turn contributed to the elucidation of a problem which still awaits its final solution. The main questions concerning amyloid are its chemical nature, source and mode of formation, and the reasons for its deposition in the tissues. It seems likely that the amyloid protein is

not a uniform substance. Its main mass consists of a protein which stains regularly and intensely with Congo red. This does not mean that all amyloid deposits are of the same chemical composition; it only indicates that a Congo red-positive fraction is present in all amyloid deposits. Hass and Schultz found that the largest protein fraction in amyloid is a globulin which later combines, apparently, with Congo red. Pure globulin is Congo red-negative and precipitates of antigens and antibodies give only a slight Congo red reaction. Amyloid protein is doubly refractile in polarised light after Congo red staining (Ladewig, Romhanyi). One can conclude, therefore, that Congo red is deposited in preformed structures of a submicroscopical nature within the amyloid.

The iodine and iodine-sulphuric acid reactions disappear after pepsin-HCl treatment; the Congo red staining remains. The iodine reaction returns if the tissue is put into blood plasma from a patient with amyloid.

Twenty-five years ago we showed that there is an increase of serum globulin in the amyloid mouse. At that time, the globulin estimation was performed only once at the end of each experiment. New micro- methods for protein estimation made us repeat these experiments, estimating the proteins at intervals during the course of each. In blood from the tail vein of the mouse total protein and globulin were estimated nephelometrically (Zeiss photometer). The results show that after one injection of casein in sodium hydroxide there is a fall in the albumin and a rise in the globulin. These changes are more intense after 5 injections. After 15 injections a difference is seen in the diseased and control animals in that the amyloid group shows a more marked fall in albumin and an irregular rise in globulin. The unaffected animals show a straight and continuous rise in globulin and only a small fall in albumin.

Amyloid formation seems to depend on the variations of the plasma proteins. For this reason we used several other micro- methods for their estimation; (1) the Weltmann coagulation band, (2) fractional precipitation after Butler and Montgomery and (3) Tiselius's electrophoresis.

In normal mice the heat-coagulation limit of serum (Weltmann) lies between tubes 3 and 6 ($CaCl_2$ dilutions 0·4-0·25 per cent.). Collectively, in 103 animals, the absolute limits were found to lie between 3 and 7 ($CaCl_2$ 0·4-0·2 per cent.), 45 per cent. being at 5 (0·35 per cent.). After 5 injections the coagulation limit lies at 0 in 40 per cent. of the experimental animals. After 10 injections 40 per cent. show a coagulation limit at 2 ($CaCl_2$ 0·45 per cent.), the curve consequently showing a shift to the right. The curve, however, does not return to the normal picture, though showing a tendency to do so. After 15 injections, *i.e.* at the end of an experiment, it becomes obvious that the coagulation limit of the animals with amyloid is lower than that of the unaffected animals.

The next method applied for protein estimations was that of Butler and Montgomery, modified into a nephelometric micro- method. Precipitation of the globulins occurs at a concentration of 1·3-2·3, that of the albumins at a concentration of 2·3-3·0. After 5 injections one sees a steep rise, indicating an increase of globulin. There is a still higher rise after 10 injections, but the stability of the increasingly formed globulins is diminished. The albumin figures fall. After 15 injections one finds new protein fractions in the region of the albumins as well. Fibrinogen was not estimated, as the nephelometric method could not be used for that purpose. For the time being we have found no difference in fibrinogen content between the amyloid animals and those that remained healthy.

The shortening of the Weltmann band and the increase of globulin in the region of the euglobulins indicate an increase in β-globulins if one compares the results with the reaction types described by Wuhrmann.

Electrophoresis shows similar results, namely a fall in albumin and an increase in β-globulins after the first and second series of injections and an increase in γ-globulins after 15 injections, an increase which probably allows

one to differentiate between diseased and unaffected animals. After 20 injections the β-globulins still remain increased while the γ-globulins fall below normal. These observations agree well with the behaviour of the Weltmann band, the increase of which is equal to an increase of γ-globulins.

As to the origin and formation of amyloid, we believe that its protein constituent is not of a specific chemical nature but a part of the plasma protein. Whether it is a para-protein due to faulty plasma protein formation we do not yet know. Two processes appear to be of importance : the disturbance of the colloidal stability of the plasma and the formation of new plasma proteins of α, β and γ types. In many cases amyloid is the result of antigen-antibody reactions.

Following on the serological investigations we tried to ascertain if there is any relation between nutrition and amyloidosis. Using a variety of food-stuffs, we found that certain proteins such as plasma proteins have a protective power against amyloidosis.

Finally we tried to correlate our findings with morphological changes and elicited the following facts. The spleen is always enlarged. The liver and spleen show mesenchymal-cell proliferation : unless this mesenchymal reaction occurs there is no amyloid formation. The increase of endothelial cells per sq. mm. can be counted and used as an exact measure of the reaction. A further criterion is the change in the liver cell itself. There is an increase in the number of liver cells (hyperplasia) per sq. mm. from the 7th to the 14th day, and this is more marked in animals fed with oats and plasma protein than in those fed with oats only. Mitoses, regarded in this connection as resulting from the death of other cells, are more numerous in oats-fed animals than in those which have additional plasma. The mitoses disappear earlier in animals which have added plasma protein in their food. This probably means that the latter group suffers less from the injections of casein in sodium hydroxide because of their power to develop liver-cell hyperplasia. The animals fed only on oats show numerous mitoses for a longer period and develop a less pronounced liver-cell hyperplasia as judged by the liver-cell numbers and liver weight.

Hyperplasia is followed by hypertrophy, which represents the final compensation of the disturbance. These changes have been studied by measuring nuclear volume and comparing it with the number of cells and the quantity of cytoplasmic nucleotides as estimated by the methyl-pyronin method. In normal animals one regularly finds three classes of nuclei and a medium amount of nucleotides in cytoplasm and nuclei. Hyperplasia leads to an increase of small nuclei with a decrease of the larger ; it is combined with the appearance of mitoses and a decrease in nucleotides. In hypertrophy one finds that in all classes of nuclei the nuclear volume is increased, and there is nucleotide formation in both cytoplasm and nuclei as evidence of new protein formation. The number of cells per unit volume is decreased.

All our experiments have in common humoral, mesenchymal and parenchymal reactions and indicate that amyloid degeneration does not occur provided all these respond together in an orderly fashion in relation to time and quantity. Amyloid may show itself, however, when there is disturbance of the above-mentioned responses, a disturbance which may be conditioned by either exogenous or endogenous factors.

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NON - LIPID RETICULO - ENDOTHELIOSIS WITH DIABETES INSIPIDUS : REPORT OF A CASE WITH ESTIMATION OF POSTERIOR PITUITARY HORMONES

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(PLATES CXVI and CXVII)

THIS case of non-lipid reticulo-endotheliosis with hypothalamic involvement is of interest both on account of the rarity of the condition and because there was clinical evidence at first of disturbance of posterior pituitary function, followed later by general hypopituitarism.

We have used the term " non-lipid reticulo-endotheliosis " in accordance with the views of Schafer (1949), who discussed the relationship between this condition, the Schüller-Christian syndrome and Letterer-Siwe disease.

CASE REPORT

Clinical history

A male child, born 29th August 1937, developed normally until February 1942 (age 4½) when he had some abdominal pain and vomiting which persisted until June. He then developed some diarrhœa as well and was admitted to hospital. During this illness it was first noticed that he drank a great deal. No definite cause for the abdominal pain or diarrhœa was found and there was no jaundice. Diabetes insipidus was diagnosed and controlled with pitressin tannate. There was considerable loss of weight at this time but his condition improved under treatment and he was discharged from hospital on 30.9.42.

He remained well and happy, being seen occasionally as an out-patient, until 10.7.44, when he was admitted to hospital for dental extractions on account of oral sepsis, with Vincent's angina. The diabetes insipidus was still well controlled with pitressin tannate. He was able to read comfortably and did so very well. The visual field and optic discs appeared normal and he was

intelligent. He appeared a little under-developed for his age (nearly seven) : this was regarded as significant.

He was still very well when seen in July 1945 but by October 1946 obesity was developing and he was suffering clinically from hypopituitarism. On 25.11.46 he was admitted to hospital for liver biopsy because the liver was felt to be enlarged. At laparotomy there was a fine cirrhosis which histologically was considered to be of portal type. The spleen was not palpable, and X-ray examination of the skull and of the whole of the skeleton showed no evidence of deposits of any kind in the bones. The vault of the skull appeared well developed and the pituitary fossa was normal in shape but the facial bones and mandible were considerably under developed. The teeth still remaining in the jaw indicated a normal degree of development for the child's age. The epiphyses of the long bones corresponded with those of a child aged about 5, his actual age at this time being 9 years. The sugar tolerance curve, blood cholesterol, serum sodium, plasma chloride and serum inorganic phosphate were all within normal limits.

On 3.11.47 he was again admitted to hospital because of abdominal pain, chiefly after food. He complained of sleeping more than usual. There was slight jaundice and considerable obesity. He was slow in his movements, but his face, though expressionless, was intelligent. His height was 41 in., his weight 56 lb., the normal figures for this age being 52 in. and 67 lb. No lymph-node enlargement was palpable. The diabetes insipidus was still controlled by pitressin tannate. X-ray examination of the bones again showed no sign of tumour deposit. An X-ray of the chest showed some blurring of both lung fields and some increase in shadowing above the right diaphragm.

Blood count. Red cells 3·08 million ; Hb. 40 per cent. ; C.I. 0·65 ; white cells 5800, with normal leucocyte distribution ; reticulocytes 3 per cent. ; platelets normal.

A sternal puncture gave no evidence of arrested development of either leucocytes or red cells and no abnormal cells were seen. B.P. 125/90. Later, the jaundice increased and ascites developed and he became comatose. Râles developed at the lung bases and he died on 8.12.47. There was no fever until the terminal illness.

Summary of autopsy

The autopsy was performed 14 hours after death. A very obese little boy 107 cm. in length and looking about 5 years of age. Moderate jaundice. No skin lesions except a few fresh hæmorrhages. Some ascites. A collateral venous circulation had developed around the *liver*, which was cirrhotic and jaundiced as a result of the presence of large gall stones in the intrahepatic bile ducts. These obstructed particularly the main left duct and the left lobe of liver was almost completely atrophied. *Gall-bladder* fibrosed but contained no stones. Common duct patent.

Spleen large (375 g.), with smooth tense capsule and dark greyish firm pulp ; Malpighian bodies inconspicuous. The appearance, except for a general greyness, was that of chronic venous congestion and the splenic vein was dilated.

Lymph nodes around the pancreas, along the lesser curvature of the stomach and in the portal fissure were greatly enlarged, soft, discrete and yellowish brown on section. There was no other glandular enlargement.

PLATE CXVI

NON-LIPID RETICULO-ENDOTHELIOSIS
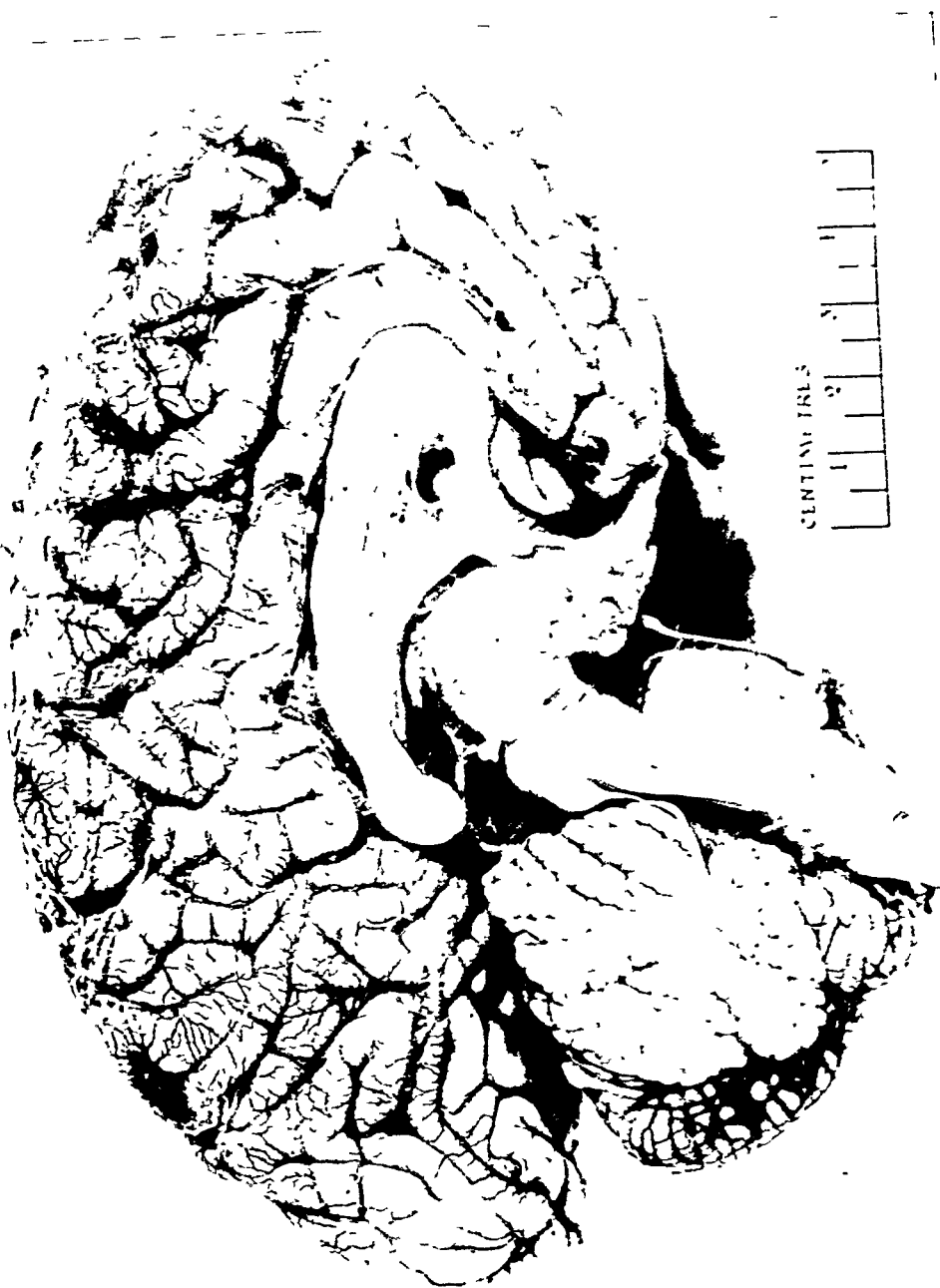
FIG. 1.—Mesial section of brain, showing the hypothalamic lesion as a rather well-defined dark grey area.

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Bone marrow in shaft of femur mostly fatty : a little red marrow at centre, increasing above.

Œsophageal veins prominent.

The *stomach* appeared healthy but a subacute ulcer and a few small acute peptic ulcers were present in the *duodenum.* The *jejunum* and *ileum* appeared normal, with no enlargement of the lymphoid follicles. On the posterior wall of the *cæcum* there was a localised area of grey infiltration about 1 cm. in thickness : this did not involve the mucosa.

The *upper respiratory tract* was healthy, with no active otitis, but there was a terminal bronchopneumonia.

Kidneys, ureters, bladder and *genital organs* were normal in gross appearance, the *testes* being in the scrotum.

Of the *endocrine* organs the *pituitary* appeared normal in size. After careful dissection the anterior lobe weighed 320 mg., the posterior lobe 16·5 mg.. the latter being thus extremely small. The *thyroid* was almost imperceptible, consisting of a thin sheet of tissue some 3 mm. in thickness. There was no gross fibrosis but the outline of the gland was very indistinct and it could not be dissected away from the surrounding structures. The *parathyroids* were not identified because a large horizontal block of the larynx was taken for histological examination of the remnants of the thyroid. The *adrenals* together weighed 4·4 g. and were extremely small and flat. *Thymus* atrophied.

Bones. There were no gross deformities and no defects. Epiphyses not enlarged. Skull of normal thickness and consistency.

Brain. Meninges normal. The only abnormality was a solid tumour occupying the hypothalamic region. It was greenish when fresh, but granular and yellow in the centre, and was thought to be a craniopharyngioma (fig. 1). There was no involvement of the optic chiasma and no obstruction of the ventricular system.

Histology

Hypothalamic tumour. What was thought to be a craniopharyngioma proved to be a granulomatous lesion with some free cholesterol crystals in the interstices and an infiltration with large acidophil histiocytes showing occasional mitoses. Some of these in the centre of the lesion, where the yellowish patch was seen in the gross, had formed giant cells with up to twenty nuclei in a given section and foamy cytoplasm (fig. 2) ; some had phagocytosed brown pigment which gave the Prussian blue reaction. A frozen section showed that the few foamy histiocytes contained sudanophil isotropic lipid, presumably neutral fat : the other histiocytes contained little or no lipid. The majority of the mononuclear histiocytes had a diameter of approximately 8-14 microns. Occasional eosinophil leucocytes were present.

Spleen. The pulp was full of similar histiocytes but the general splenic structure was maintained and the Malpighian bodies were not involved. Reticulin fibrils were increased around the histiocytes.

Lymph nodes. The enlarged abdominal lymph nodes (fig. 3) were full of histiocytes which had distended and blocked all the lymph sinuses and had also invaded the stroma to some extent. These histiocytes were similar to those seen in the hypothalamus and in the spleen. A few contained cell debris that had been phagocytosed and some had brownish pigment which gave the Prussian blue reaction. A few eosinophil cells were seen and there was also an occasional small focal necrosis.

Liver. This was grossly scarred as a result of the biliary obstruction and in the connective tissue around the more severely damaged areas there was infiltration by histiocytes. There was no suggestion that the histiocytic infiltration was the cause of the liver lesion.

Pancreas. In some sections of the pancreas there were areas of histiocytic invasion. The islets showed no abnormality.

Cœcum. The affected portion of the wall observed naked eye was infiltrated with histiocytes. Eosinophil leucocytes were more numerous in this lesion than elsewhere.

Lungs. The pneumonia was characterised by some hæmorrhage and by infiltration of the alveolar walls and particularly the alveolar spaces with histiocytes, which in this situation had become markedly phagocytic. Very few polymorphonuclear leucocytes were seen.

Pituitary. The anterior lobe of the pituitary was unfortunately slightly damaged in the process of dissection, which was undertaken before fixation. In consequence there was some drying of the cells at the periphery of the gland and a certain amount of shrinkage was evident throughout. None the less it was quite clear that there were very few acidophil cells present and that, even allowing for shrinkage, those present were considerably smaller than normal. On comparing sections of this gland with others from a normal boy aged 9 years it was estimated that there were approximately 5 times as many acidophil cells per field in the normal gland as there were in the gland from the present case. As a result of the shrinkage it was not possible to make an accurate count of the basophil and chromophobe cells. There was no suggestion of histiocytic invasion of the anterior pituitary and the fragment of the posterior lobe which was present in some sections also contained no histiocytes.

Thyroid. This was so small that horizontal sections had to be cut through the whole of the larynx so as to include what remained of the thyroid. It was represented only by a few minute collections of atrophied acini and groups of shrunken cells without acinar arrangement. These were surrounded by fibrous tissue invaded by large numbers of histiocytes.

Adrenals. These showed considerable atrophy of the cortex

PLATE CXVII

NON-LIPID RETICULO-ENDOTHELIOSIS
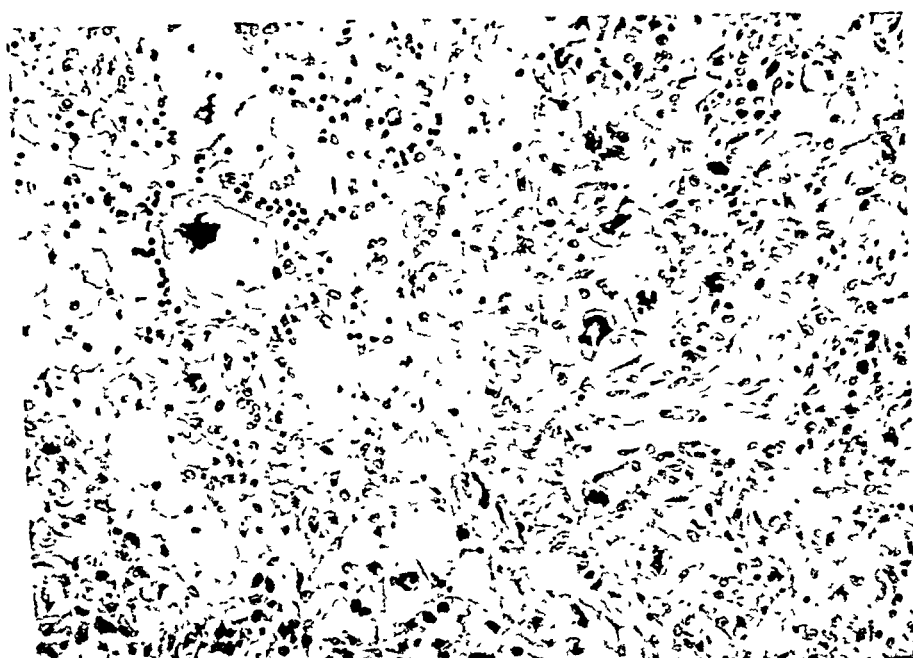
FIG. 2.—Centre of hypothalamic lesion, showing foamy histiocytes and giant cells, two of them in mitosis, and background of non-lipid containing histiocytes.   ×200.
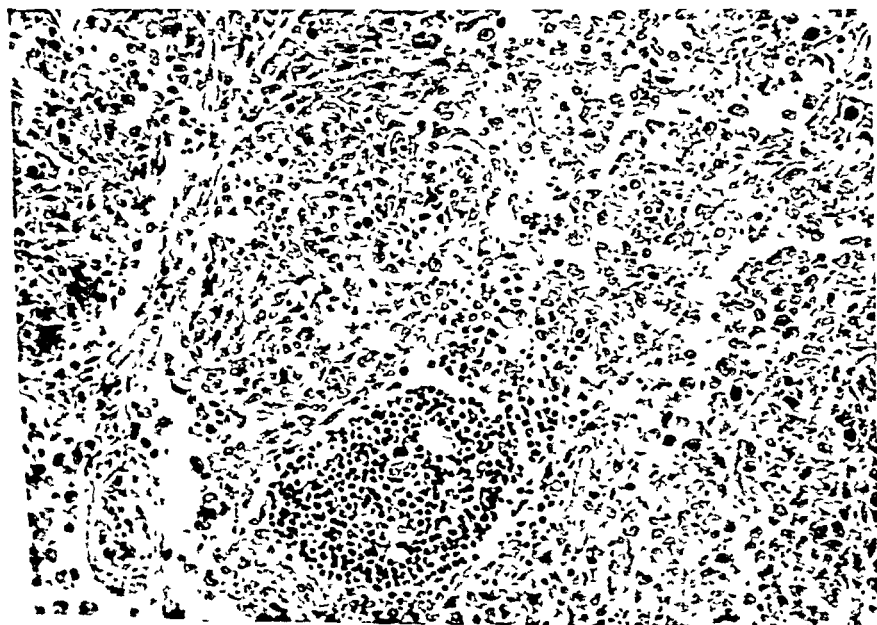
FIG. 3.—Abdominal lymph node, showing sinuses and parenchyma over-run with acidophil histiocytes.   ×200.

involving all layers but particularly the zona fasciculata. No histiocytic invasion was seen.

Thymus. Sections through the fatty tissue in the region of the thymus failed to show any sign of thymic tissue.

Testes. These appeared normal for a child of this age.

Estimation of posterior pituitary hormones

The posterior lobe, after dissection, was placed in acetone and extracts prepared as described by Heller and Zaimis (1949). The antidiuretic activity of the extracts was estimated by means of intravenous injections into non-anæsthetised rabbits (Heller, 1940-41). Virgin guinea-pig uteri were used for the assay of oxytocic activity. Posterior pituitary extract (British Drug Houses) was used as the standard preparation. The table shows the results compared with controls from 14 normal adults and 15 newborn infants. It will be noted that the weight of the posterior lobe of the present subject, a boy aged 10 years, was only that of a new-born infant and contained only minute quantities of antidiuretic and oxytocic principles.

TABLE

Mean size and hormone content of normal human posterior pituitary glands contrasted with the figures for the case of diabetes insipidus

	Wt. of anterior lobe (mg.)	Wt of posterior lobe (mg)	Wt. of posterior lobe as percentage of whole gland	Dry wt. of posterior lobe (mg.)	Percentage solids in posterior lobe	Antidiuretic activity		Oxytocic activity	
						mU./ mg. dry gland	mU./ posterior lobe	mU./ mg. dry gland	mU./ posterior lobe
Adults (14)	435 ± 33 8	131 ± 6·5	24·5 ± 1·82	19·8 ± 1·4	14·9 ± 0 53	761 ± 58	14570 ± 1580	747 ± 45	13850 ± 1085
Newborn infants (15)	60 ± 5·9	15 ± 1·6	23·6 ± 2·22	2·4 ± 0·2	15·8 ± 1·38	166 ± 25	375 ± 40	150 ± 38	387 ± 20
Case of diabetes insipidus aged 10	312·8	16·5	5·3	2·9	17·5	≈ 1·7	≈ 5	<1·7	<5

The normal figures are from the paper of Heller and Zaimis (1949)

DISCUSSION

It is significant that there was no sign of invasion of either the anterior or the posterior lobe of the pituitary by the reticulo-endotheliosis ; it is therefore reasonable to assume that the diabetes insipidus was produced by the hypothalamic lesion. The hormone content was found to be less than 1 per cent. of normal. We have found no other reference to hormone estimation in the neurohypophysis of human material from cases of diabetes insipidus, but the very low hormone content agrees with experimental findings in animals subjected to section of the pituitary stalk (Fisher and Ingram, 1936 ; Hare *et al.*, 1941 ; Hickey *et al.*, 1941).

An interesting feature of the present case is the clinical evidence of disturbance of function of the anterior lobe of the pituitary two

years after the onset of diabetes insipidus. This is not entirely unexpected, since Globus *et al.* (1947) have demonstrated that lesions in the hypothalamus may give rise not only to diabetes insipidus but also to disturbance of anterior pituitary function, together with atrophy of other endocrine glands. In the present case it was noted that there was a deficiency of acidophil cells in the anterior lobe of the pituitary and extreme atrophy of both the thyroid and the adrenal cortex. It is true that the remnants of the thyroid were invaded by histiocytes but there were none in the adrenals and we considered the atrophy of both to be due to extraneous factors. Both the pituitary and the thyroid changes may have contributed to the cessation of growth.

It may be noted that the diabetes insipidus persisted up to the time of death in spite of the severe degree of hypothyroidism and of cortical atrophy of the adrenals. It has been claimed (Globus *et al.*) that hypothalamic lesions do not produce diabetes insipidus unless the thyroid, and probably also the adrenal cortex, are normally active.

In the absence of any clear proof of nervous connections between the hypothalamus and the anterior hypophysis the occurrence of changes in the anterior lobe may perhaps be attributed to disturbance of its blood supply by the lesion in the hypothalamus, since Green and Harris (1949) have shown that, in the rat, blood flows from the region of the tuber cinereum through the hypophysio-portal circulation to the anterior lobe of the pituitary. The involvement of the hypothalamus by massive reticulo-endotheliosis may well have interfered with these vascular channels.

The relationship between Schüller-Christian disease and Letterer-Siwe disease is discussed by Schafer (1949), who agrees with other writers, including Wallgren (1940) and Mallory (1942), that there are transitional forms between the lipid-containing lesions of the former disease and the non-lipid lesions of the latter. The term " non-lipid reticulo-endotheliosis " is therefore to be preferred for cases such as the present which lack several of the characteristic manifestations of Letterer-Siwe disease.

SUMMARY

A case is reported of a boy aged 10 who developed normally until the age of 5, when diabetes insipidus appeared. Two years later growth ceased and he became obese.

The diabetes insipidus was due to involvement of the hypothalamus by a non-lipid reticulo-endotheliosis.

Both the antidiuretic and oxytocic hormones of the posterior pituitary were estimated and found to be greatly reduced.

There was a deficiency of acidophil cells in the anterior pituitary and extreme atrophy of the thyroid and of the cortex of the adrenals.

The relation of the hypothalamic lesion to these autopsy findings is discussed.

Our thanks are due to Professor C. Bruce Perry for the use of his clinical notes, to Dr Eleanor J. Zaimis for assistance with the hormone assays and to Mr G. Rogers for the photographs.

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PROGNOSIS IN CUTANEOUS AND OCULAR MALIG-NANT MELANOMA : A STUDY OF 222 CASES

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(PLATES CXVIII-CXXIV)

MALIGNANT melanoma, on the whole a highly malignant tumour, is also of very variable histological structure and clinical course. Indeed it is still not generally realised that the prognosis is not uniformly bad and that there is a marked difference in outlook in skin and eye cases. Moreover it is only within recent years that prepubertal melanoma of the skin has come into prominence in the literature. It would seem that true malignant melanoma rarely exists before the age of puberty, the prepubertal lesion being usually, at most, only a transition towards malignancy. Doubtful or transitional melanomata are by no means rare and are not confined to children, although usually met with in the younger age groups. These cases offer great difficulty in histological diagnosis even to the experienced histopathologist.

In the present study, all cases of melanoma submitted to histological examination in the Leeds General Infirmary over the 23-year period 1925-47 have been reviewed. The series is a continuation of that reported by Gleave (1929), who covered the previous 15-year period. The cases collected were (1) those in which a definite histological diagnosis of malignant melanoma had been made, (2) doubtful cases, where nævus-cell moles had been regarded as suspicious, and (3) obviously malignant lesions which had been regarded as possibly melanomatous. Of a total of 234 cases, 86 were ocular, 148 cutaneous and mucosal. Of the latter, 115 were in group 1, 29 in group 2 and 4 in group 3. The histological sections were next examined, in consultation with Professor Stewart, without reference to the original diagnosis and report. The following changes were then made in the grouping ; three cases in group 1 were considered to be malignant but not melanomatous and were rejected, and five in group 2 were considered to be entirely benign and also rejected. Of the four cases in group 3 two are probably not malignant melanomata, the other two may well be genuine but are not included in the series. Six of group 1 were transferred to group 2 and three of

group 2 to group 1. These changes result in 109 of the skin cases now being regarded as malignant melanomata and 27 as doubtful.

Including the 86 eye cases, all of which are regarded as frankly malignant, there is a total list of 222 cases of which 208 are from the Leeds General Infirmary, 11 from other Leeds hospitals and 3 from private sources. Two hundred and fourteen of the cases have been followed up by personal communication with the patient, if alive, or with his relatives and private doctor.

MALIGNANT MELANOMA OF THE SKIN AND MUCOUS MEMBRANES

In this series of 109 confirmed cases 105 have been followed up.

Age distribution. The disease was rare under the age of 20 and became frequent in the 5th to the 8th decades, 78 per cent. of the cases falling into the 40-79 age group. Pack *et al.* (1947*b*, 862 cases) found the large majority in the 35-70 age group and emphasised that

TABLE I

*Age distribution at the time of operation in 109 cases of
malignant melanoma of the skin and mucosœ*

Age periods	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89
Number	1	1	9	12	24	17	24	20	1

Youngest 8 years, oldest 84 ; average 52·6 years

of their 15 prepubertal cases none metastasised and all survived indefinitely ; they would classify this group separately. In the present series only one case considered to be frankly malignant on histological grounds was encountered below the age of 19—that of a child of 8 who was alive and free from recurrence 3 years later.

Sex distribution. This showed no significant difference—60 females (55 per cent.) and 49 males (45 per cent.) and is in accord with previous findings.

Sites of occurrence. These are shown in the following list :—

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Head and neck (excluding eye)  .      .      .      .      . 28 (26 per cent.)
     Face      .      .      .      . 24 (22 per cent.)
Trunk   .      .      .      .      .      .      .      . 30 (28  ,,    ,, )
Extremities   .      .      .      .      .      . 43 (39  ,,    ,, )
     Upper limb      .      .      . 14 (13 per cent.)
     Lower limb      .      .      . 29 (27  ,,    ,, )
          Foot .    . 15 (14 per cent.)
Other sites   .      .      .      .      .      .      . 6
     Nasopharynx     .      .      . 1
     Vagina   .      .      .      . 2
     Cervix   .      .      .      . 1
     Anus and rectum .      .      . 2
No primary found    .      .      .      .      .      . 2
                                                          ___
          Total .   .      .      .      .      .      . 109
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The most common sites were thus the face (22 per cent.) and the lower limb (27 per cent.). The difference between the upper and lower limb incidence is striking and it should be noted that no less than half of the lower limb tumours were on the foot.

Origin in a pre-existing mole. Forty-six (49 per cent.) of 94 cases where the information was available gave a history of a pre-existing mole, present either from birth (20 cases) or for several or many years. Daland and Holmes (1939, 128 cases) obtained a corresponding figure of 44·6 per cent. and Pack *et al.* (1947b, 862 cases) recorded 50 per cent., while some authors have reported even higher figures (Ackerman, 1948, 75 cases, 61 per cent.; Driver and MacVicar, 1943, 60 cases, 80 per cent.) and some lower (Coley and Hoguet, 1916, 91 cases, 39·6 per cent.; Horwitz, 1928, 49 cases, 36·7 per cent.; Gleave, 1929, 22 cases, 36 per cent.).

Role of trauma. Trauma has frequently been regarded as an exciting factor in the production of malignant melanoma and probably plays a part in some cases. In this series there was a history of trauma in 18 cases (16·5 per cent.). Pack *et al.* (1947b) found a history of trauma in not more than 20 per cent. and in most of these it was considered a doubtful factor : on the other hand they emphasise the importance of chronic irritation. The common mole on the lower limb, especially on the foot, may well acquire malignant properties by virtue of the trauma to which it is constantly exposed. Hewer (1935), in a series of 42 malignant melanomata of the skin in natives of the Sudan, found that 28 were situated on the foot, 17 being on the sole. He comments that this high incidence, especially on the sole of the foot, in people who walk bare-foot in a country abounding with thorns and sharp stones is fairly strong evidence for the significance of trauma in their causation. The factor of trauma is also probably not negligible in moles on the face. It has already been noted that the commonest sites for malignant melanoma, both in this and in other authors' series, are the face and lower limb, particularly the foot.

Cases coming to autopsy. Eleven of the skin cases were examined *post mortem*, and multiple generalised secondary deposits found in all but one—the malignant melanoma of nasopharynx. Secondary deposits in the brain were particularly common, being present in six of the nine cases with metastases in which the brain was examined : Peller (1941) stated regarding his autopsy findings that it was amazing how frequently metastases of melanoma were found in the brain.

Prognosis

Seventy of these cases had a possible 5-year or longer survival time, of which 28·6 per cent. survived the 5 years. The average survival period of the 70 cases was 3½ years from the time of operation and 12 were still alive with an average survival of 10½ years. Fifty-

eight were dead : they had survived for an average of $2\frac{5}{12}$ years. de Cholnoky (1941) took the average of all authors with large series and obtained a figure of 19·2 per cent. of 5-year arrests. Recently Pack *et al.* (1947*b*), in an analysis of 595 unselected cases with a possible 5-year survival, found the over-all 5-year salvage to be 9·7 per cent. This low figure may be accounted for by the fact that 64 per cent. of the total of 862 cases available for analysis had recurrent tumours when first seen : they had received prior treatment elsewhere.

TABLE II

Survival rate in malignant melanoma of the skin and mucosæ

Survival period (years) .	1	2	3	4	5 *	10 †
No. of possible cases .	95	88	80	76	70	48
Percentage surviving .	83·2	58	42·5	34·2	28·6	12·5

* Four had developed recurrence during this time
† All were free from recurrence

The prognosis was worse in the older age groups, a greater percentage being dead, with a smaller proportion of 5-year survivals, but the fatal cases had approximately the same survival times in the various age periods. Lesions on the trunk had the poorest prognosis, a greater percentage being fatal and the average survival time less than for other sites. Upper limb lesions had a good prognosis but only five cases were available. Head and neck and lower limb cases had a similar prognosis.

Prognosis in subungual melanoma. Dawson (1925) laid stress on the gravity of melanoma of the nail or nail fold and on the rapidity of development, intense pigmentation and speedy breaking down of the regional glands related to this site. Pack and Adair (1939), on the other hand, stated that the percentage of cures was higher in this site than in any other location and that amputation of the involved region was usually curative if no metastasis had occurred. In the present series of 109 cases there were six subungual lesions (5·5 per cent.) and three situated on terminal phalanges without being related to the nail bed, making nine cases in all where amputation of the digit would be the treatment of choice. Of the six subungual cases five were males and the average age was 66. Two of the three non-subungual cases were males and the average age was 54. The average age of the nine cases was 62. One of the subungual group was situated on the big toe and five on fingers. Two of the non-subungual cases were situated on the big toe and one on a finger, making a total of three on the big toe and six on fingers. It is interesting to note that seven of the nine cases, including all three on the big toe, gave a definite history of trauma. Eight cases were treated by complete and one by partial amputation of the digit, five being preceded bv biopsy.

Dissection of the regional glands was carried out at the same time in only four cases where the glands were palpable. In two (both subungual cases) they were appreciably enlarged and proved to be invaded by growth; one of these patients died nine months later; the other is still alive after $2\frac{1}{12}$ years. Only slight glandular enlargement was present in the other two (both non-subungual), but microscopically one showed early invasion and the patient died eleven months later; the other patient is still alive after $5\frac{3}{12}$ years. Of the five cases where the glands were not removed at the time of amputation only two (both subungual) are still alive after $13\frac{3}{12}$ and $4\frac{1}{12}$ years, the latter six months after excision of local glandular metastasis. The other three patients died from metastases after ten months (non-subungual), $1\frac{7}{12}$ years and one year (subungual) respectively; the last two had developed local glandular metastases.

The prognosis in this group of cases, irrespective of whether the tumour is in fact subungual, does not appear good and is certainly no better than lesions elsewhere as judged from this small series. Five of the nine were dead with an average survival period of only one year and two of the surviving cases have developed local glandular metastasis. It would seem that the glands tend to be invaded early, particularly where the tumour is situated on the toe, and here trauma may well be a factor in inducing rapid spread. Microscopic deposits in the glands are quite likely to have been present in the first instance in the three cases which subsequently developed glandular enlargement, as amputation would preclude the possibility of any local recurrence which might have undergone metastasis to the glands. Recurrence in the stump did not occur in any of these cases. The danger therefore lies in the regional glands, which should be excised in every case whether palpable or not. If there are no metastases in the glands amputation should give good results. The outlook in cases with microscopic metastases in non-palpable glands is unpredictable, as no large series has as yet been reported. Should the regional glands be enlarged and invaded the prognosis is usually hopeless and more extensive surgical measures than simple excision of the glands is the only hope.

Prognosis in relation to glandular involvement and local recurrence. In 7 cases the primary tumour had been removed elsewhere and the patient came under observation either with a local recurrence or with enlarged lymph glands. Of the 109 cases 7 were considered hopeless, and of these, 4 were biopsied only; in 2 the primary was removed, in the other it was examined histologically only *post mortem*. In a further 77 cases the primary alone was removed, and in 2 others the glands were removed though no primary was found. The primary and the regional glands were removed together, or within six weeks, in the remaining 23 cases. To deal with this last group first; in 18 cases the glands were clinically enlarged, and, in all but two, proved to be invaded on histological examination. The average survival period from the time of operation for 13 of the 16 cases where the

eight were dead : they had survived for an average of $2\frac{5}{12}$ years. de Cholnoky (1941) took the average of all authors with large series and obtained a figure of 19·2 per cent. of 5-year arrests. Recently Pack *et al.* (1947*b*), in an analysis of 595 unselected cases with a possible 5-year survival, found the over-all 5-year salvage to be 9·7 per cent. This low figure may be accounted for by the fact that 64 per cent. of the total of 862 cases available for analysis had recurrent tumours when first seen : they had received prior treatment elsewhere.

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glands were invaded was $1\frac{7}{12}$ years, the other three being still alive but only followed up over a short period. Five of these cases developed further lymphadenopathy and again had glands excised, and it is interesting to note that these include some of the longest survival periods in the 16 cases, namely 5, $3\frac{9}{12}$, 3, $1\frac{11}{12}$ and $1\frac{2}{12}$ years. The two cases in which the glands were not invaded are still alive after $5\frac{3}{12}$ and $2\frac{11}{12}$ years. In only five cases was a block dissection of the regional glands carried out where these were not palpable clinically, and in two there were microscopic secondary deposits. Three of the five are still alive after $16\frac{4}{12}$ years (glands invaded, fig. 3), 5 years and $2\frac{4}{12}$ years (glands not invaded), while the other two died after $2\frac{9}{12}$ years (glands invaded : developed local recurrence) and $1\frac{3}{12}$ years (glands not invaded but terminal subcutaneous metastases).

Recurrence without general spread may be either local, or metastatic in the regional glands. Growth developing between these sites is not known to have occurred in this series apart from a terminal popliteal gland metastasis in one case ; subcutaneous deposits of random distribution and not related to this area have been encountered terminally in a few cases. Local recurrence occurred in at least 19 (22 per cent.) of a total of 88 possible cases and was excised in 15. Its development was not more frequent in cases with palpable glandular metastasis in the first instance.

In 18 (25 per cent.) of the 73 cases followed up in which the primary alone was excised, enlarged regional glands developed at an average interval of $1\frac{7}{12}$ years (longest interval $4\frac{5}{12}$ years) after the primary operation, and were removed. Four recent cases are still alive but the remainder all died shortly after removal of the glands (average ten months : longest subsequent survival $1\frac{5}{12}$ years). In one of these cases the enlarged glands proved to be merely hyperplastic, although local recurrence then developed with invaded glands. This case makes three in all where glands were enlarged but not invaded. In the same group of 73 cases, but excluding four where amputation was performed, local recurrence developed in 16 (23 per cent.) and it is remarkable to note that 8 were patients included in the 18 who developed enlarged metastatic regional glands. A further case was known to have developed metastatic glands after local recurrence but they were not excised. This association indicates that the glandular metastasis in the 8 cases was in all probability due, in some at least, to the local recurrence which either developed before and was excised, or was present at the time of excision of the enlarged glands. It is also obvious that, even if block dissection of the glands had been carried out in the first instance, the growth would not have been completely eradicated. In the other ten cases microscopic deposits in the glands, although not palpable, may have been present when the primary was excised, as was indeed found in two of five cases in this series. Pack *et al.* (1945) reported that of ten axillary dissections in which there were no palpable nodes, microscopic deposits were found in five and also in

PROGNOSIS IN MALIGNANT MELANOMA

FIG. 1.—Malignant melanoma of ankle, showing deep infiltration and a nodule of growth in the subcutaneous adipose tissue. Patient still alive after 16¼ years. ×5.

FIG. 2.—Higher power view of part of fig. 1 to show fibrosarcomatous type of structure. ×60.
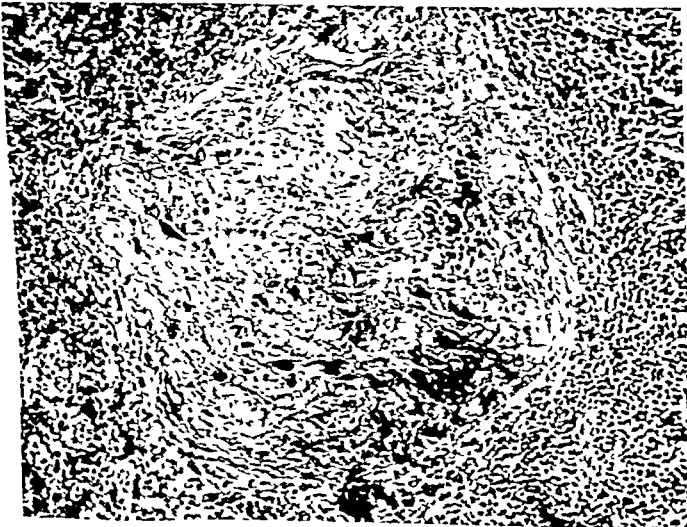
FIG. 3.—Tiny secondary deposit in non-palpable inguinal gland excised in prophylactic block dissection. Same case as figs. 1 and 2. ×100.

All sections stained with hæmatoxylin and eosin.

two of seven groin dissections. Enlarged regional glands developed in more than the 18 cases but were terminal and not excised.

Of the glands which had been excised in a total of 42 cases, 22 were inguinal, 15 axillary, 3 cervical, 1 supraclavicular and 1 submental. In the two cases where no primary was found death occurred within two months of excision of the glands. Radiation therapy was used in the treatment of many of the cases, apparently without much success.

From these results it is evident that when enlarged metastatic regional glands have developed at an interval after removal of the primary, simple excision of the glands is likely to be of little value ; the outlook, indeed, is hopeless. In the cases where regional glands were found to be enlarged and invaded in the first instance and were excised at the same time as the primary the results were little better : all proved fatal, with only a slightly longer average survival. Sampson Handley pointed out in 1907 that in cases with palpable enlargement simple excision of the glands was likely to be useless, as permeation of the lymphatic plexus of the deep fascia soon took place around the affected glands. He advised that a large area of the deep fascia be removed with the glands. Pack *et al.* (1945, 1947*a*) advocated radical excision of the primary and metastatic tumours in continuity and even fore- or hind-quarter amputation.

The prognosis also proved to be poor in the 7 cases where the primary alone was excised and in which local recurrence developed without glands. Four died from metastasis shortly after recurrence ; one died $4\frac{3}{4}$ years later from " old age " ; one is still alive 1 year after excision of a second recurrence, and the other was found to have a second recurrence which had developed 4 years after excision of the primary.

As there are only five cases in this series in which block dissection of non-palpable glands was performed at the same time as the excision of the primary tumour, it is difficult to judge the results of this operation. Three of these patients are still alive, including one with microscopically invaded glands who has survived $16\frac{1}{12}$ years (figs. 1-3). It still remains to be seen what the follow-up results of a large series of these cases would be. It may be that if the glands prove to be invaded the prognosis is bad and more radical surgery necessary. Local recurrence is an additional risk ; it occurred in at least 22 per cent. of all possible cases. The few long survivals in this series have been obtained, with one exception, solely by simple excision of the primary tumour, which implies that the tumour must have been completely localised. It is, however, not uncommon to find tiny satellite nodules of growth in the subcutaneous tissue around the primary, and in order to improve results in the other cases—the vast majority—wide local excision is essential. Of cases where the primary alone was excised 36 per cent. developed either local recurrence (23 per cent.), enlarged metastatic regional glands (25 per cent.)

or both, without other evidence of spread. These figures, together with the probability that in some cases the metastatic glands were the result of local recurrence, suggest that more cases might be saved if the primary growth were more widely excised. Wide excision of the primary is probably the most valuable measure in the treatment of malignant melanoma of the skin with non-palpable glands. In addition, the regional glands should be dissected out en bloc—preferably a month later—in all cases wherever possible, although, if they are found to be invaded, the prognosis without more radical surgery is as yet unpredictable.

Causes of death. Of the cases followed up 70 were dead, and from a careful analysis of all the data in each case the conclusions in table III were reached regarding the cause of death. If only cases with the possibility of 5 years survival or longer are considered the difference is but slight. The patients who have probably not died from malignant melanoma show a higher average age at death and a much longer average survival after operation than those dying of malignancy, which is in favour of their not having died from metastasis. In the cases where

TABLE III

Analysis of causes of death in 70 fatal cases of malignant melanoma of the skin and mucosæ

	No. of cases	Average age at death	Average survival after operation (years)
Total cases dead . .	70	58	$2\frac{3}{12}$
Probably dying of malignant melanoma	60 (86 per cent.)	56	$1\frac{11}{12}$
Probably not dying of malignant melanoma	4 (6 „ „)	74	$5\frac{4}{12}$
Cause of death not known .	6 (8 „ „)	70	$3\frac{2}{12}$

the cause of death could not be determined, some have probably died from malignancy and some from other causes. Twenty of the 70 cases in which there was a possibility of 5 years' survival or longer exceeded the 5-year limit and 12 were still alive after periods up to $21\frac{7}{12}$ years. Only three or perhaps five cases died from malignancy more than 5 years after excision of the primary growth and, of the five, four had developed glandular secondaries during these 5 years. It would therefore appear that if a patient survives 5 years with no enlargement of glands or local recurrence there is a very good chance of his being entirely free from growth.

Pathology

Ulceration was present in 70 per cent. of the primary lesions, and, as one would expect, the largest tumours tended to have the worst prognosis. The degree of pigmentation was divided as far as

PROGNOSIS IN MALIGNANT MELANOMA

FIG. 4.—Nævoid type of malignant melanoma. Regional glands, although not palpable, were invaded and the patient died from metastases $2\frac{1}{2}$ years later. ×60.

FIG. 5.—Doubtful lesion, showing deep, sharply outlined downgrowth into the dermis (to right). Patient alive after $10\frac{1}{2}$ years. ×90.

PROGNOSIS IN MALIGNANT MELANOMA

FIG. 6.—Doubtful lesion, with mass of irregular pigmented cells invading subcutaneous adipose tissue. Patient alive after $10\frac{1}{12}$ years. ×60.

FIG. 7.—Doubtful lesion of only moderately cellular spindle-cell structure, with arrangement in interweaving sheaves. Patient alive after $8\frac{5}{12}$ years. ×45.

possible into four grades, but no definite correlation with prognosis could be established.

An attempt was made to classify the tumours according to cell type but this proved to be by no means easy. They were grouped into round- (epithelioid-) cell, spindle-cell and mixed-cell types. The epithelioid-cell type appears to have the worst prognosis, but the series is too small to permit of reliable conclusions being drawn. In general, from a practical standpoint, grading on cell type by itself is probably of little value. In two varieties of cutaneous malignant melanoma, however, histological characters seem to be of importance in evaluating prognosis. The more important is the " fibro-sarcomatous " group of spindle-cell tumours. These, histologically, while undoubtedly malignant, are relatively bland-looking as compared with the average malignant melanoma, suggesting a lower grade of malignancy. In the more typical examples (figs. 1-3) they are not particularly cellular, the cells being much elongated and arranged in whorls. Of the eight cases in this group, five were still alive $16\frac{4}{12}$, 9, $1\frac{5}{12}$, $\frac{10}{12}$ and $\frac{5}{12}$ years after operation and three had died after $7\frac{9}{12}$ years (from " diffuse and multiple melanotic sarcoma "), $6\frac{2}{12}$ years (from " old age ") and $2\frac{4}{12}$ years (from " chronic nephritis "). The prognosis was good, as only one case had apparently died from malignancy and then only after nearly 8 years survival. The other variety, the " anaplastic " group of nine cases, had, on the contrary, a bad prognosis. Five were dead with an average survival after operation of only $1\frac{9}{12}$ years, the other four are recent cases.

Depth of local cutaneous infiltration is probably of less value in prognosis than degree of cellularity and anaplasia, as very superficial tumours in this series have caused early death, while deeply infiltrating tumours of less cellular type (fig. 1) have resulted in long survival.

DOUBTFULLY MALIGNANT MELANOMATA OF THE SKIN OR NÆVUS-CELL MOLES SHOWING TRANSITIONS TO MALIGNANCY

This group consists of 27 cases selected on purely histological grounds from those reported over the last 23 years as malignant melanomata or as nævus-cell moles in which there was a considerable or even a strong suspicion of malignancy. The constitution of the group, classed on review as " doubtful," has already been described : 25 of them have been followed up.

TABLE IV

Age distribution at the time of operation in 27 cases of nævus-cell moles regarded as showing transitions to malignancy

Age periods	0 9	10-19	20 29	30 39	40-49	50 59	60 69	70-79	80-89
Number . .	9	2	6	4	2	2	1	1	0

Youngest $\frac{10}{12}$ years, oldest 74 ; average 25·2 years

Age distribution.   This is shown in table IV.

While the lesion may occur at any age it was much more common in the younger age groups and gradually became infrequent in the older age groups.   Forty per cent. were in children under the age of 12. This is in striking contrast to the malignant group (table I).

Sex distribution.   This is predominantly female—20 females and 7 males.

Sites of occurrence.   These are shown in the following list.

		Male	Female
Face	16 (59 per cent.) .	5	11
Trunk	3 (11 ,, ,,) .	1	2
Lower limb	7 (26 ,, ,,) .	0	7
Site unknown	1 (4 ,, ,,) .	1	0
		7	20

The face was thus much the commonest site, and the face and lower limb together made up 85 per cent. of the cases (59 per cent. on the face and 26 per cent. on the lower limb as compared with 22 per cent. and 27 per cent. respectively in the frankly malignant group).

Prognosis

It is of great importance to know what the prognosis is likely to be in cases where the pathologist is undecided as to whether a mole is malignant or not.   In this group, extending over a 23-year period, all but 2 of the 25 cases followed up were found to be alive and well.   One of the two patients who died was said to have been perfectly well $7\frac{2}{12}$ years after excision of the lesion and died after $8\frac{6}{12}$ years, at the age of 82.   The cause of death could not be ascertained but it may well have been other than metastasis.   The second patient, a woman of 68, died two months after removal of a lesion from the toe, apparently from cerebral thrombosis, and there was no sign of recurrence.   In only one of the 23 surviving cases has there been local recurrence, namely, a child of 6 in which a nævus of the conjunctiva recurred 6 years after excision ; neither the initial lesion nor the recurrence was frankly malignant histologically.   If cases prior to 1943 are taken, that is, those with a possibility of 5-year or longer survival, there are 14 cases and the average survival time to date is 10 years.

Pathology

Histologically (figs. 5-12), these cases cannot be passed over as simple nævus-cell moles nor can they be definitely classed as malignant. It must be the experience of most pathologists that there is a considerable group of intermediate cases of this kind, showing transitions between the simple nævus cell and the malignant melanoma

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Age distribution at the time of operation in 27 cases of nævus-cell moles regarded as showing transitions to malignancy

Age periods .	0 9	10-19	20 29	30-39	40 49	50-59	60 69	70-79	80-89
Number . .	9	2	6	4	2	2	1	1	0

Youngest $\frac{10}{12}$ years, oldest 74 , average 25·2 years

2 L

Age distribution. This is shown in table IV.

While the lesion may occur at any age it was much more common in the younger age groups and gradually became infrequent in the older age groups. Forty per cent. were in children under the age of 12. This is in striking contrast to the malignant group (table I).

Sex distribution. This is predominantly female—20 females and 7 males.

Sites of occurrence. These are shown in the following list.

		Male	Female
Face	16 (59 per cent.) .	5	11
Trunk	3 (11 „ „) .	1	2
Lower limb	7 (26 „ „) .	0	7
Site unknown	1 (4 „ „) .	1	0
		7	20

The face was thus much the commonest site, and the face and lower limb together made up 85 per cent. of the cases (59 per cent. on the face and 26 per cent. on the lower limb as compared with 22 per cent. and 27 per cent. respectively in the frankly malignant group).

Prognosis

It is of great importance to know what the prognosis is likely to be in cases where the pathologist is undecided as to whether a mole is malignant or not. In this group, extending over a 23-year period, all but 2 of the 25 cases followed up were found to be alive and well. One of the two patients who died was said to have been perfectly well $7\frac{3}{12}$ years after excision of the lesion and died after $8\frac{6}{12}$ years, at the age of 82. The cause of death could not be ascertained but it may well have been other than metastasis. The second patient, a woman of 68, died two months after removal of a lesion from the toe, apparently from cerebral thrombosis, and there was no sign of recurrence. In only one of the 23 surviving cases has there been local recurrence, namely, a child of 6 in which a nævus of the conjunctiva recurred 6 years after excision ; neither the initial lesion nor the recurrence was frankly malignant histologically. If cases prior to 1943 are taken, that is, those with a possibility of 5-year or longer survival, there are 14 cases and the average survival time to date is 10 years.

Pathology

Histologically (figs. 5-12), these cases cannot be passed over as simple nævus-cell moles nor can they be definitely classed as malignant. It must be the experience of most pathologists that there is a considerable group of intermediate cases of this kind, showing transitions between the simple nævus cell and the malignant melanoma

PROGNOSIS IN MALIGNANT MELANOMA

FIG. 8.—Doubtful lesion, probably malignant. A low-power section of the whole lesion, showing the cellularity and deep downward extension. Patient alive after $7\frac{5}{12}$ years. ×15.

FIG. 9. — Higher power view of downward extension in fig. 8, showing the cellular pigmented masses invading the subcutaneous adipose tissue. ×65.
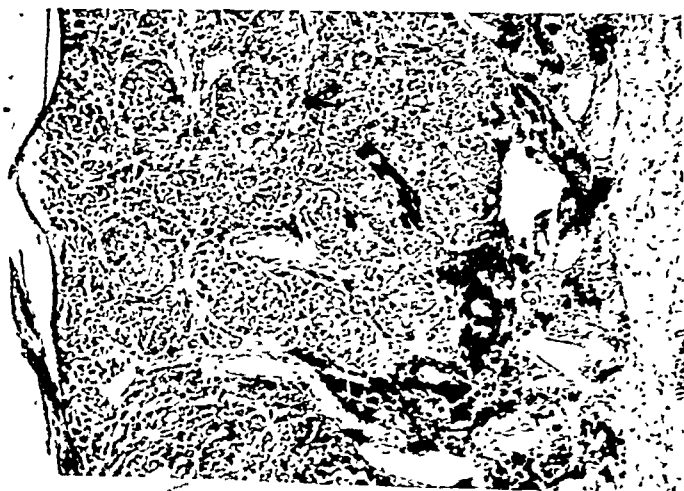
FIG. 10.—Doubtful lesion, showing solid alveolar groups of epithelioid cells and heavy pigmentation, mainly within phagocytes, on the deep aspect of the tumour (to right). Patient alive after $1\frac{2}{12}$ years. ×80.

cell. It was impossible to say in some instances whether the lesions were in process of becoming malignant, or had, in fact, become early malignant melanomata. The difficulty in diagnosis is certainly great, but it is indeed striking that the cases picked out solely on histological grounds as doubtful or transitional should prove, when followed up, to have a long survival period, with no conclusive evidence of malignancy.

As far as could be gathered, superficial ulceration was present in only one of these 27 cases : this contrasts with the malignant melanomata, the majority of which (70 per cent.) were ulcerated. Size is probably an important factor here, as the lesions in the doubtful group were all small. Pigmentation was not prominent, 19 being slightly, 7 moderately and one heavily pigmented. Malignant melanoma is well known for the diverse cell patterns that it may assume, but this feature is even more noticeable in the doubtful group. Some of the lesions were entirely superficial, others extended rather deeply.

No specific changes can be described whereby a simple nævus-cell mole is so altered that it comes within the doubtful group, but in general the important changes are in the morphology and arrangement of the cells, which become irregular, hyperchromatic, and may be increased in size and assume an epithelioid character or may become spindle shaped. A solid alveolar grouping of large epithelioid cells and deep infiltration by anaplastic nævus cells are both of significance. The general nævoid character of the lesion is usually apparent, but not always, and this nævoid appearance is not infallible by itself. as one of the malignant group, while showing this feature (fig. 4), was otherwise frankly malignant. The regional glands from this case were removed a month later and, although they had not been palpable, showed infiltration of the peripheral sinuses by tumour cells ; the patient developed a local recurrence $2\frac{4}{12}$ years later and died from generalised secondary deposits $2\frac{9}{12}$ years after excision of the primary.

It will be seen that while there is a gradual merging of the simple nævus-cell mole with malignant melanoma, there is a substantial intermediate group, histologically doubtful, in which the prognosis is very good. While stressing this difficulty of histological diagnosis, I wish to point out that unless the lesion is frankly malignant on general histological grounds the prognosis in a large proportion of cases should be good.

Treatment. In all 27 cases in this group simple excision was the only treatment carried out. This proved to be adequate and there is no indication for prophylactic dissection of glands. As a precautionary measure, however, the patient should be examined periodically.

Malignant melanoma in children

It is well known that in infants and children many of the moles which are said to be histologically malignant prove to be clinically

benign. As already mentioned, this has been recently emphasised by Pack *et al.* (1947*b*), who would classify their prepubertal melanomata separately, since, of 15 cases, none metastasised and all survived indefinitely. Another recent paper by Spitz (1948), who also stressed this point, went further and considered whether there was any histological difference between the melanomata of children and adults ; it was concluded that, in most cases, differentiation could not be made with certainty.

Of the total of 222 cases dealt with in this paper, 12 were under the age of 19 years, that is, children, as the range was $\frac{10}{12}$ year to 11 years. Eleven of the lesions occurred on the face and one on the buttock. In Spitz's series of 13 cases of childhood melanoma, five were on the face, one on the trunk, two on the upper extremity, and five on the lower extremity. In the present series 11 of the 12 cases were followed up and all proved to be alive and well after intervals up to $22\frac{6}{12}$ years ; in only one was there recurrence, the child of 6 with a conjunctival lesion which recently recurred 6 years after excision. All but one of Spitz's cases were alive after intervals ranging up to 13 years ; the exception died from metastases.

It is significant that in the present series of childhood lesions only one was considered frankly malignant histologically, the others being all in the doubtful or transitional group.

OCULAR MALIGNANT MELANOMA

Of the 86 malignant melanomata of the eye 80 came from the Leeds General Infirmary during the period 1925-47, an average of 3·5 cases per year. At Moorfields from 1871 to 1925 there was an average of 6 per year (Davenport, 1927). Eighty-four of the 86 cases have been followed up. The eye was enucleated in all cases but one, in which a tumour of the iris was excised locally.

Age distribution. These tumours seldom occur under the age of 20 and the incidence in this series was highest in the sixth and seventh

TABLE V

Age distribution at the time of operation in 86 cases of malignant melanoma of the eye

Age periods	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89
Number	0	0	2	13	10	30	23	7	1

Youngest 23 years, oldest 82 ; average 53·7 years

decades. Callender *et al.* (1942, 1418 cases) found the greatest number of patients in the sixth decade and Benjamin *et al.* (1948, 248 cases) found an average age of 53·71 years.

PLATE CXXII

FIG. 11.—Doubtful lesion which, although cellular and deeply infiltrating, was relatively bland-looking under higher magnification. No sign of recurrence after six months. ×8.
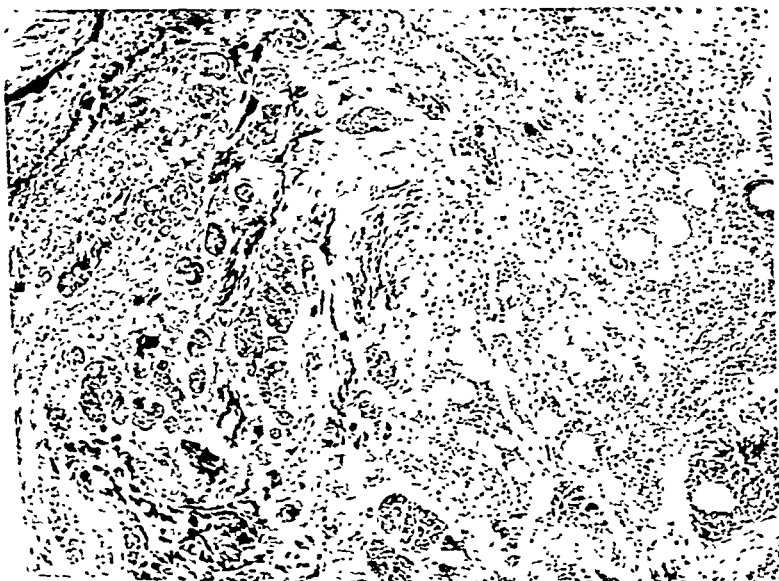
FIG. 12.—Doubtful lesion, showing fairly innocent-looking structure on right, with more malignant-looking heavily pigmented solid alveolar tissue on left. The patient, a woman aged 68, died from cerebral thrombosis two months after operation. ×55.

Sex distribution. The tumours were of equal incidence in the two sexes, as was also found in 1550 cases by Callender *et al.*

Sites of occurrence. The commonest site is the choroid coat, with a few cases arising in the iris or ciliary body, or in various combinations

TABLE VI

Sites of 86 cases of malignant melanoma of the eye

Site	Choroid	Iris	Ciliary body	Choroid and C B	Iris and C B	Orbit	Not known
Number .	66	5	3	5	1	1	5

of these three sites. The case in which the tumour arose in the orbit was an encapsulated orbital melanoma, apparently primary, recorded by Foster in 1944.

Role of trauma. There was a history of trauma in only four cases (4·7 per cent.). In one, the tumour was said to have followed a perforating wound of the eye by a needle. Another was interesting in that a foreign body had destroyed the sight of an eye. and 14 years after the eye had been removed (it was not examined pathologically) the patient returned with a large melanomatous mass in the orbit.

Cases coming to autopsy. Six cases were examined *post mortem*, of which three were of special interest. One was a case of diffuse meningeal melanoma secondary to a flat or diffuse malignant melanoma of the choroid ; visceral metastases were also present. The other two had secondary deposits in the skin following orbital recurrence after previous enucleation of the affected eye. Multiple generalised metastases were present in five cases ; the other, a patient aged 82. died from bronchitis. As in the skin cases, secondary deposits in the brain were common, occurring in three or possibly four of the five cases with metastases.

Prognosis

There were 56 cases with a possible 5-year survival and of these 62·5 per cent. survived the 5 years and 21 were still alive. The average survival period of this group, including those still alive, was $7\frac{6}{12}$ years at the time of follow-up.

The prognosis in malignant melanoma of the eye is good ; in this series there were 62·5 per cent. of 5-year and 39·4 per cent. of 10-year survivals. Recent authors have obtained comparable figures. Callender *et al.*, in a large series of 500 malignant melanomata of the choroid and ciliary body, followed for 5 years or longer, found 52 per cent. of 5-year and 34 per cent. of 10-year survivals. Benjamin *et al.* (1948) record the remarkable figures of 80·7 per cent. of 5-year and 73·4 per cent. of 10-year survivals. These are based, however, almost solely on communications with the Registrar General and, as

the authors point out, hinge on the reliability of his method of follow-up. Considerably poorer results were recorded by Pack *et al.*

Survival rate in malignant melanoma of the eye

Survival period (years) .	1	2	3	4	5	10	15	20
No. of possible cases .	78	72	68	65	56	33	12	3
Percentage surviving .	93·6	80·6	69·1	61·5	62·5	39·4	41·7	33·3

(1947*b*), who found 12·8 per cent. of 5-year survivals in 58 cases. Of earlier authors, Lawford and Collins (1890-93) recorded only 25 per cent. of 79 patients alive three years after enucleation of the affected eye.

There was a slightly worse prognosis in the older age groups in this series, a larger percentage being dead, although fatal cases had approximately the same survival time in each age group. Recent authors have also found an increase in mortality with advancing age.

Malignant melanoma of the iris is said to occur at an earlier age than melanoma of the choroid. Kronenberg (1938) found, in nine cases, an average age of 40·3 years as compared with 52·6 years for melanoma of the choroid and quotes Fuchs's figures as 31 years for 16 cases and 42·2 years for his whole series of uveal melanomata. Benjamin *et al.* recorded five cases with an average age of 50·4 years. In the five iris cases of the present series the average age proved to be higher than that of the choroidal melanomata, namely 66 years. The prognosis is said to be better than that of the choroidal tumours, probably because, by virtue of their site, they tend to be recognised earlier than other ocular melanomata. Four were treated by enucleation of the eye and the other by local iridectomy. Only one patient, a man aged 76, has died, and then only after nearly 5 years' survival, from a " seizure " ; three have now survived more than 5 years. Callender *et al.* recorded only three deaths in 32 cases observed for 5 years or longer.

Only three cases of malignant melanoma of the ciliary body were available for study ; these showed no notable difference in age incidence or prognosis from the choroidal melanomata.

Recurrence within the orbit following enucleation of an eye for melanoma occurred in seven cases (8·3 per cent.), after intervals ranging from 5 months to $6\frac{2}{12}$ years. From the time of presentation at hospital with recurrence the patients lived an average of only $1\frac{4}{12}$ years and all died within 2 years. Four cases were treated by exenteration of the orbit, the other three by radiation only : the prognosis proved uniformly bad. Subcutaneous deposits followed the orbital recurrence in two cases. Lawford and Collins in a series of 79 cases found local recurrence in seven (8·86 per cent.).

Causes of death. The number of fatal cases was 45 and, as in the skin melanomata, the causes of death were analysed and the conclusions in table VIII reached. If only cases with a possibility of 5 years' survival or longer are considered the results are practically identical.

TABLE VIII

Analysis of causes of death in 45 fatal cases of malignant melanoma of the eye

	No. of cases	Average age at death	Average survival after operation (years)
Total cases dead . .	45	62	4$\frac{4}{12}$
Probably dying of malignant melanoma	32 (71 per cent.)	60	3$\frac{4}{12}$
Probably not dying of malignant melanoma	5 (11 ,, ,,)	72	9$\frac{4}{12}$
Cause of death not known .	8 (18 ,, ,,)	63	5$\frac{1}{12}$

Lawford and Collins found, in their follow-up of 79 cases, that 40 were dead, 26 (65 per cent.) from metastasis, with an average duration of life after operation of 2$\frac{4}{12}$ years.

A 5-year survival following enucleation of an eye for malignant melanoma is clearly too early to judge whether or not a patient is free from growth. Of the 56 cases followed for 5 years or more, seven died from metastases more than 5 years after enucleation of the eye, the longest interval being 10$\frac{8}{12}$ years. Cases have been recorded of patients developing metastases after even longer intervals in malignant melanoma of the eye, but these are very rare. Cairns's (1922-23) case is often quoted, where a deposit appeared in the scapula secondary to a melanoma of the eye removed 18 years previously. It is therefore less easy in the case of the eye than in the skin to fix any time limit when the patient can be considered to have a reasonable chance of being free from growth. Survival for 10 years without recurrence should, in the great majority of patients, give a very good chance of their being entirely free from growth ; Martin-Jones (1946) came to a similar conclusion. In the present series only one patient died from metastases more than 10 years (at 10$\frac{8}{12}$ years) after enucleation of the affected eye and yet thirteen survived 10 years and nine were still alive after periods up to 22$\frac{1}{12}$ years.

Pathology

I think the size of the tumour in melanoma of the eye does bear some relationship to prognosis. Size was estimated as far as possible from descriptions of the tumours and from sections. As might be expected, the larger tumours (fig. 13) had the worst prognosis. There were 13 cases with a possibility of 5 years' survival or longer where the

tumour occupied more than $\frac{1}{4}$ of the globe on cross section, and of these only 5 (38 per cent.) survived 5 years or more : all are now dead. This figure of 38 per cent. compares with 77 per cent. for the smaller tumours (fig. 14) excluding the " diffuse." group. The " diffuse " malignant melanomata (fig. 15) infiltrate the uveal tract without forming a definite tumour and have a bad prognosis ; according to Parsons (1905) they are rare, and extra-ocular extension occurs frequently and relatively early. There were six cases of diffuse melanoma in this series and all died from metastasis in an average period of $1\frac{10}{12}$ years from the time of enucleation of the eye. This may be compared with an average of $3\frac{4}{12}$ years for the whole series of deaths from metastasis in melanoma of the eye. Extra-ocular extension was present in five of the diffuse cases and the remaining (6th) case showed infiltration of the sclera by melanin-laden indeterminate cells, whether malignant or non-neoplastic macrophages it was impossible to say.

Shape, apart from the diffuse type, whether flattened, nodular or " keyhole," did not appear to influence prognosis, nor did the presence or absence of breaching of the membrane of Bruch.

Pigmentation was graded in the same way as in the skin tumours, but in some cases grading was difficult owing to the degree of pigmentation differing in different parts of the growth (fig. 13). A better prognosis was apparent in the less pigmented tumours, as there were 86 per cent. of 5-year survivals in grade I (21 cases), compared with 38 per cent. in grades II (13 cases), III (10 cases) and IV (6 cases) combined (total 29 cases). McGregor and Hill (1943) and Benjamin et al. (1948) also found a significantly lower mortality associated with low pigment content.

Prognosis in malignant ocular melanoma in relation to cell type. On a basis of cell type Callender divided malignant melanoma of the eye into four categories, namely (1) spindle-cell types A and B, (2) fascicular type, (3) epithelioid-cell type and (4) mixed-cell type. All authors are agreed that there are spindle-cell, round-cell and mixed-cell types and I have found it difficult to go much beyond this simple classification with the material available. It will be seen from table IX that there is a significant difference between the epithelioid- and spindle-cell types in the number of 5-year survivals ; the epithelioid-cell type certainly appears to have the worst prognosis. Callender et al., who analysed the cell type in 500 cases followed 5 years or longer, also found the epithelioid-cell type to be the most malignant, a finding on which, indeed, there is general agreement. Special grouping into anaplastic and fascicular (perithelial) types does not appear to be of great value, although the anaplastic type does seem to have a slightly worse and the fascicular type a slightly better than average prognosis.

Extra-ocular growth was present in twelve cases (16 per cent.) and was associated with a high mortality, all cases but one being

FIG. 13.—Malignant melanoma of eye almost filling the globe and showing patchy distribution of pigment. Patient died from metastasis $2\frac{5}{12}$ years after enucleation of the eye. ×3·3.

FIG. 14.—Small malignant melanoma of eye. Patient still alive after $14\frac{5}{12}$ years. ×4.
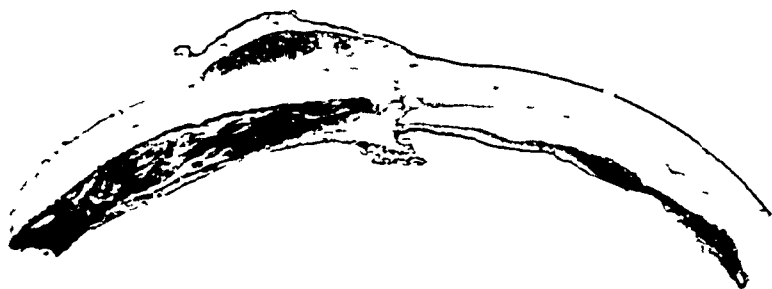
FIG 15 —Diffuse type of malignant melanoma of eye, involving choroid, ciliary body and iris, and showing an extra ocular deposit   Patient died from metastasis $1\frac{1}{2}$ years after enucleation of the eye.   × 7.

FIG. 16 —Malignant melanoma of eye showing infiltration of the sclera and early extra ocular extension.   Patient still alive after $6\frac{1}{2}$ years   × 60.

fatal on an average in $1\frac{11}{12}$ years; the exception (fig. 16) was still alive after $6\frac{4}{12}$ years. Scleral infiltration by itself, whether by definite

TABLE IX

Prognosis of malignant melanoma of the eye in relation to cell type

	Round or epithelioid cell	Spindle cell	Mixed cell
No. of cases (75) . . .	15	56	4
No. of cases with possible 5-year survival or longer (48)	10	37	1
Percentage of 5-year survivals .	20	70	0

growth or by melanin-laden cells of indeterminate character, did not have such a uniformly bad prognosis as the cases with definite extra-ocular growth, but the prognosis was still bad. Where there is infiltration of the optic nerve without other evidence of spread the outlook would appear to be good. Two cases with infiltration of the optic nerve were alive after $3\frac{4}{12}$ years and $4\frac{7}{12}$ years. A third died after four months but in this case extra-ocular growth was present in addition. Martin-Jones was also of the opinion that involvement of the optic nerve was not a bad prognostic sign; of his eleven examples, only two died within 3 years and then also showed extra-ocular growth. It certainly appears that the factor responsible for early death in these cases is the extra-ocular extension.

SUMMARY

This study is concerned with 222 cases of malignant melanoma of the eye, skin and mucous membranes and includes nævus-cell moles of doubtful malignancy. The cutaneous and mucosal lesions were classified histologically into 109 malignant melanomata and 27 doubtful nævus-cell moles.

There was a 28·6 per cent. 5-year- and a 12·5 per cent. 10-year-survival rate in the malignant melanomata of the skin as compared with the much better prognosis in the eye cases of 62·5 and 39·4 per cent. respectively.

Diagnosis in the doubtful or transitional group is difficult, but the cases in which the lesions were so labelled histologically proved to have almost indefinite survival and, while occurring at all ages, were especially frequent in children and the younger age groups. True malignant melanoma is probably rare in children.

Prognosis was worse in the later age groups in malignant melanoma of both skin and eye, and in the former, lesions on the trunk had a higher mortality than those in other gross sites. Subungual melanoma did not have a good prognosis; it would seem that the glands tend to be invaded early.

Where the regional glands were found to be enlarged and invaded, either at the time of excision of the primary growth or later, simple excision of the glands proved to be of little value and more radical surgery was the only hope, as the average subsequent survival was only $1\frac{7}{12}$ years and ten months respectively. In 73 followed-up cases the primary alone was excised and 36 per cent. developed either local recurrence (23 per cent.), enlarged metastatic regional glands (25 per cent.) or both, without other evidence of spread. These figures, together with the probability that in some cases the metastatic glands were the result of local recurrence, suggests that more cases might be saved if the primary growth was more widely excised. Wide excision of the primary is probably the most valuable measure in the treatment of malignant melanoma of the skin with non-palpable glands. In addition, wherever possible, the regional glands should be dissected out—preferably a month later—in all cases, although the prognosis without more radical surgery if they are found to be invaded, is as yet unpredictable. Cases in the doubtful group require only simple excision of the primary growth.

If a patient with malignant melanoma of the skin survives for 5 years after its excision, without glandular involvement or local recurrence, there is a good chance of his being entirely free from growth ; the corresponding period in the eye cases is 10 years.

Trauma probably plays a definite part as an exciting factor in the ætiology of some skin melanomata, especially those of the foot : in eye cases a history of trauma is infrequent.

Two histological variants of skin melanoma, the fibrosarcomatous and the anaplastic, are recognised : they had better and worse than the average prognosis respectively.

There is some correlation between size of tumour and prognosis in ocular melanoma ; the large tumours tend to have the worst prognosis, except that the diffuse melanoma of the uveal tract has also a poor prognosis. In the eye melanomata, higher mortality was apparent in the more deeply pigmented tumours and in those of epithelioid-cell type. The average survival time in cases where the enucleated eye showed extra-ocular growth was only $1\frac{11}{12}$ years. Involvement of the optic nerve is not necessarily a bad prognostic sign. There was orbital recurrence in 8·3 per cent. of cases and the average survival period from the time of diagnosis was $1\frac{4}{12}$ years.

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The rabbits were anæsthetised with Nembutal and ether and the fluid was injected into the trachea, after surgical exposure, with a syringe and a hypodermic needle of wide bore.   During the injection and until they had recovered consciousness, the rabbits were made to lie upon their right sides.   When lesions were subsequently found in the lungs they were usually high up on the right side.   In the case of the animals injected with their own amniotic fluid, the hysterectomy and the injection were carried out during one and the same period of anæsthesia.

In some of the earlier experiments the rabbits were killed by a blow on the neck, but this sometimes caused terminal aspiration of blood.   Later the animals were all killed by an overdose of nembutal intravenously.

Results

Rabbit amniotic fluid experiments

Rabbits injected with amniotic fluid from other rabbits.   There were 4 rabbits in this group, and they were killed at intervals of 2-4 days after the injection.   In two which had received 2 c.c. of sterile fluid no lesions were found.   In one, killed 3 days after the injection of 2 c.c. of fluid slightly contaminated with *Staphylococcus aureus*, there was collapse of the lung with macrophage and polymorphonuclear infiltration and perivascular lymphatic infiltration.   In the fourth animal, killed 4 days after the larger injection of 5 c.c. of sterile fluid, there were small scattered areas of collapse with some macrophage and polymorphonuclear infiltration.

Rabbits injected with their own amniotic fluid.   There were 3 animals in this group, and a control injected with sterile normal saline.   An animal which was killed 2 days after the intratracheal injection of 10 c.c. of its own sterile amniotic fluid showed fairly extensive subpleural collapse with some polymorphonuclear infiltration, some eosinophilic debris and nuclear remnants in the collapsed alveoli, and perivascular lymphocytic infiltration.   The control animal, which received 10 c.c. of sterile 0·9 per cent. sodium chloride solution and was killed after 2 days, showed changes which were similar but less extensive and less severe.   The similarity of the lesions is shown in figs. 3-6.   In the lungs of 2 rabbits killed 3 and 4 days after receiving 5 c.c. of their own amniotic fluid—sterile but slightly bloodstained— there were only small inconspicuous areas of collapse with a few hæmosiderin-containing macrophages.

Human amniotic fluid experiments

Rabbit amniotic fluid was never obviously contaminated with meconium and contained less-cellular material than human.   To study the effect of solid constituents, a series of rabbits was injected intratracheally with human liquor amnii.   Some of the specimens of fluid were autoclaved to assure sterility, and, in some, artificial meconium staining was obtained by grinding up 0·3 g. of sterile human meconium in 15 c.c. of the sediment from a specimen of human

liquor amnii. Preliminary experiments showed that sterile meconium was an irritant and would cause an abscess when injected subcutaneously. Of the various constituents of meconium which were tested by intratracheal injection, bile salts caused the most acute inflammatory reaction in the lungs, producing fatal pulmonary œdema with hæmorrhage and leucocytic emigration.

Human amniotic fluid was injected intratracheally in 14 animals. Of these, 5 which received 2 c.c. of fluid free from cells and solid material, were killed from 3 to 14 days later. Although some of this fluid was contaminated with bacteria, changes in the lungs were slight or absent. When a lesion was found, it consisted of slight collapse and was associated with perivascular lymphocytic infiltration.

In a group of 3 animals, cells from human amniotic fluid were injected. In one case these were suspended in amniotic fluid; in the other two, they were washed free from amniotic fluid with saline, and were suspended in saline. In all three, sterility was obtained by autoclaving the material to be injected. The amount of fluid used ranged from 2 to 10 c.c., and the rabbits were killed after intervals of 3, 4 and 9 days. After 3 and 4 days there was some polymorphonuclear infiltration. In all of them the reaction, which was associated with collapse, showed many macrophages, some of which had coalesced to form foreign-body giant cells (fig. 7).

The remaining 6 animals each received 2 c.c. of thick amniotic deposit artificially stained with meconium and slightly contaminated with Gram-positive cocci and Gram-negative bacilli. The contamination was thought to have occurred during the collection of the fluid. In an animal killed 10 hours after such an injection there was collapse of alveoli with abundant polymorphonuclear infiltration. Keratinised squamous cells were recognised in the alveoli (fig. 8) and there was perivascular lymphocytic infiltration. In the animal killed after 5 days the changes were still marked but now the keratinised cells had disappeared and many giant cells were present (fig. 9). There were also signs of absorption by lymphatics, as indicated by cellular infiltration and dilatation of the sub-pleural lymphatics (fig. 10). The remaining animals were killed after 8, 11, 15 and 32 days. No keratinised cells could be found in the lungs, which showed gradual resolution of the inflammatory lesions, but even in the animals killed after 32 days small scattered areas of collapse with foreign-body giant cells could still be found on careful microscopic examination, and macrophages containing bile pigment were found in an area of lymphoid tissue.

DISCUSSION

The chief difficulty in interpreting these results is that observations upon the lungs of adult animals may not be applicable to the lungs of fœtal animals. In post-fœtal life, fluid of even the most bland nature, although it is rapidly absorbed from the lungs (Courtice and

Phipps, 1946-47), seems to cause collapse and inflammation in propor-
tion to the amount of fluid introduced.   This is in accord with the
findings of Miller *et al.* (1949) in dogs.   Though these workers reported
that rabbits showed little inflammatory response to saline, the rabbit
injected with saline in the present series showed distinct changes.

In the case of human amniotic fluid the cellular debris has an
additional irritant effect and can induce a foreign-body reaction.
The injected cells cease to be recognisable in the lungs in the course
of about 5 days.   The inflammatory changes generally are enhanced
by meconium staining of the amniotic fluid.   The presence of
perivascular round-cell infiltration and of dilated sub-pleural lymphatics
suggests that lymphatic absorption is involved in the removal of the
amniotic material from the lungs.   These lesions are usually not severe
and resolve completely in a few weeks.   They produce no obvious
illness in the animal.

The interest of these observations is in their relation to the lungs
of the fœtus *in utero*.   The fœtal lung is certainly capable of an
inflammatory response, as is proved by the presence of inflammatory
areas in the lungs of some stillborn babies (MacGregor, 1939).   It
might reasonably be presumed that, unless there is some marked
difference between fœtal and adult lungs, the inhalation of liquor
amnii into the alveoli of the fœtus would lead to a low-grade
inflammatory response.   If such inflammation were not very wide-
spread the baby might show no clinical evidence of the lesion, which
would disappear within the first few days or weeks of life.   If such
inflammation were very extensive, the baby would be liable to die
at birth so that the pulmonary lesions could be found microscopically.
A search has been made for foreign-body giant-cell reactions in the
lungs of several hundred cases of stillbirth and neonatal death.   Such
a reaction is very rare, and it is not mentioned by Johnson and Meyer
(1925), Farber and Sweet (1931), Helwig (1933), Warwick (1934, 1937),
MacGregor (1939), or Dick and Pund (1949),   Since chronic reactions
are not found, it seems probable that amniotic fluid enters the lungs
of the baby only when it is in distress.   The acute reactions sometimes
found in the lungs may well have developed during the course of a
protracted labour.   That the lungs of the newly born baby are capable
of a foreign-body reaction is shown in fig. 11.   This presents a picture
of multinucleated giant cells in the lung of an infant which survived
for 4 days with many squamous cells in its alveoli.   The rarity of such
a finding indicates that few babies which have inhaled amniotic fluid
survive for any length of time, and it seems probable that a baby
inhales before birth only when it is in difficulties.

SUMMARY

An inflammatory response occurs after the introduction of saline,
rabbit amniotic fluid or human amniotic fluid into the lungs of adult

cell. It was impossible to say in some instances whether the lesions were in process of becoming malignant, or had, in fact, become early malignant melanomata. The difficulty in diagnosis is certainly great, but it is indeed striking that the cases picked out solely on histological grounds as doubtful or transitional should prove, when followed up, to have a long survival period, with no conclusive evidence of malignancy.

As far as could be gathered, superficial ulceration was present in only one of these 27 cases; this contrasts with the malignant melanomata, the majority of which (70 per cent.) were ulcerated. Size is probably an important factor here, as the lesions in the doubtful group were all small. Pigmentation was not prominent, 19 being slightly, 7 moderately and one heavily pigmented. Malignant melanoma is well known for the diverse cell patterns that it may assume, but this feature is even more noticeable in the doubtful group. Some of the lesions were entirely superficial, others extended rather deeply.

No specific changes can be described whereby a simple nævus-cell mole is so altered that it comes within the doubtful group, but in general the important changes are in the morphology and arrangement of the cells, which become irregular, hyperchromatic, and may be increased in size and assume an epithelioid character or may become spindle shaped. A solid alveolar grouping of large epithelioid cells and deep infiltration by anaplastic nævus cells are both of significance. The general nævoid character of the lesion is usually apparent, but not always, and this nævoid appearance is not infallible by itself, as one of the malignant group, while showing this feature (fig. 4), was otherwise frankly malignant. The regional glands from this case were removed a month later and, although they had not been palpable, showed infiltration of the peripheral sinuses by tumour cells; the patient developed a local recurrence $2\frac{4}{12}$ years later and died from generalised secondary deposits $2\frac{9}{12}$ years after excision of the primary.

It will be seen that while there is a gradual merging of the simple nævus-cell mole with malignant melanoma, there is a substantial intermediate group, histologically doubtful, in which the prognosis is very good. While stressing this difficulty of histological diagnosis, I wish to point out that unless the lesion is frankly malignant on general histological grounds the prognosis in a large proportion of cases should be good.

Treatment. In all 27 cases in this group simple excision was the only treatment carried out. This proved to be adequate and there is no indication for prophylactic dissection of glands. As a precautionary measure, however, the patient should be examined periodically.

Malignant melanoma in children

It is well known that in infants and children many of the moles which are said to be histologically malignant prove to be clinically

benign. As already mentioned, this has been recently emphasised by Pack *et al.* (1947*b*), who would classify their prepubertal melanomata separately, since, of 15 cases, none metastasised and all survived indefinitely. Another recent paper by Spitz (1948), who also stressed this point, went further and considered whether there was any histological difference between the melanomata of children and adults ; it was concluded that, in most cases, differentiation could not be made with certainty.

Of the total of 222 cases dealt with in this paper, 12 were under the age of 19 years, that is, children, as the range was $\frac{10}{12}$ year to 11 years. Eleven of the lesions occurred on the face and one on the buttock. In Spitz's series of 13 cases of childhood melanoma, five were on the face, one on the trunk, two on the upper extremity, and five on the lower extremity. In the present series 11 of the 12 cases were followed up and all proved to be alive and well after intervals up to $22\frac{6}{12}$ years ; in only one was there recurrence, the child of 6 with a conjunctival lesion which recently recurred 6 years after excision. All but one of Spitz's cases were alive after intervals ranging up to 13 years ; the exception died from metastases.

It is significant that in the present series of childhood lesions only one was considered frankly malignant histologically, the others being all in the doubtful or transitional group.

OCULAR MALIGNANT MELANOMA

Of the 86 malignant melanomata of the eye 80 came from the Leeds General Infirmary during the period 1925-47, an average of 3·5 cases per year. At Moorfields from 1871 to 1925 there was an average of 6 per year (Davenport, 1927). Eighty-four of the 86 cases have been followed up. The eye was enucleated in all cases but one, in which a tumour of the iris was excised locally.

Age distribution. These tumours seldom occur under the age of 20 and the incidence in this series was highest in the sixth and seventh

TABLE V

Age distribution at the time of operation in 86 cases of malignant melanoma of the eye

Age periods	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89
Number	0	0	2	13	10	30	23	7	1

Youngest 23 years, oldest 82 ; average 53·7 years

decades. Callender *et al.* (1942, 1418 cases) found the greatest number of patients in the sixth decade and Benjamin *et al.* (1948, 248 cases) found an average age of 53·71 years.

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THE EFFECTS OF THE INTRODUCTION OF AMNIOTIC FLUID INTO RABBITS' LUNGS

A. H. CRUICKSHANK

From the Department of Pathology, University of Liverpool

(PLATES CXXV-CXXVIII)

THAT the fœtus is capable of intercostal and diaphragmatic movements long before full term has been amply demonstrated in sheep and rabbits by the physiological studies of Barcroft and Barron (1936-37), Rosenfeld and Snyder (1935-36), Barcroft (1941) and others. That true respiratory movements do occur *in utero* and cause amniotic fluid to enter the lungs seems also to have been demonstrated by the human experiments of Davis and Potter (1946), but, convincing as this work is, many who have studied the lungs of infants dying soon after birth still feel that such intra-uterine inhalation of liquor amnii must be abnormal.

It is certainly difficult to believe that human amniotic fluid, which is often quite turbid owing to the presence of cells, hair and other debris from the skin of the fœtus, should normally enter the fœtal lungs. Fig. 1 is a smear of the deposit which settled from a specimen of human amniotic fluid. A small bronchus with its lumen half filled with keratinised squamous cells is shown in fig. 2. Even though such material should later be absorbed from the lung or otherwise disposed of, the presence of solid debris within the bronchi of an infant might be expected to lead for a time to incomplete expansion of the lungs after birth.

The following study deals with the effects of the introduction of rabbit amniotic fluid and of human amniotic fluid into rabbits' lungs.

METHODS

Amniotic fluid was collected with sterile precautions from rabbits killed late in pregnancy, or at hysterectomy on a pregnant rabbit into whose lungs the fluid was subsequently to be introduced. It was difficult to collect the amniotic fluid completely free from blood in these experiments. The sterility of the fluid was checked by culture of a 1 c.c. sample in nutrient broth for 24 hours. Rabbit amniotic fluid was found to be much less turbid than human and only very few squamous cells could be found in it. Human amniotic fluid was obtained by puncture of the membranes in parturient women, or by paracentesis in cases of hydramnios.

The rabbits were anæsthetised with Nembutal and ether and the fluid was injected into the trachea, after surgical exposure, with a syringe and a hypodermic needle of wide bore. During the injection and until they had recovered consciousness, the rabbits were made to lie upon their right sides. When lesions were subsequently found in the lungs they were usually high up on the right side. In the case of the animals injected with their own amniotic fluid, the hysterectomy and the injection were carried out during one and the same period of anæsthesia.

In some of the earlier experiments the rabbits were killed by a blow on the neck, but this sometimes caused terminal aspiration of blood. Later the animals were all killed by an overdose of nembutal intravenously.

RESULTS

Rabbit amniotic fluid experiments

Rabbits injected with amniotic fluid from other rabbits. There were 4 rabbits in this group, and they were killed at intervals of 2-4 days after the injection. In two which had received 2 c.c. of sterile fluid no lesions were found. In one, killed 3 days after the injection of 2 c.c. of fluid slightly contaminated with *Staphylococcus aureus*, there was collapse of the lung with macrophage and polymorphonuclear infiltration and perivascular lymphatic infiltration. In the fourth animal, killed 4 days after the larger injection of 5 c.c. of sterile fluid, there were small scattered areas of collapse with some macrophage and polymorphonuclear infiltration.

Rabbits injected with their own amniotic fluid. There were 3 animals in this group, and a control injected with sterile normal saline. An animal which was killed 2 days after the intratracheal injection of 10 c.c. of its own sterile amniotic fluid showed fairly extensive subpleural collapse with some polymorphonuclear infiltration, some eosinophilic debris and nuclear remnants in the collapsed alveoli, and perivascular lymphocytic infiltration. The control animal, which received 10 c.c. of sterile 0·9 per cent. sodium chloride solution and was killed after 2 days, showed changes which were similar but less extensive and less severe. The similarity of the lesions is shown in figs. 3-6. In the lungs of 2 rabbits killed 3 and 4 days after receiving 5 c.c. of their own amniotic fluid—sterile but slightly bloodstained—there were only small inconspicuous areas of collapse with a few hæmosiderin-containing macrophages.

Human amniotic fluid experiments

Rabbit amniotic fluid was never obviously contaminated with meconium and contained less-cellular material than human. To study the effect of solid constituents, a series of rabbits was injected intratracheally with human liquor amnii. Some of the specimens of fluid were autoclaved to assure sterility, and, in some, artificial meconium staining was obtained by grinding up 0·3 g. of sterile human meconium in 15 c.c. of the sediment from a specimen of human

liquor amnii. Preliminary experiments showed that sterile meconium was an irritant and would cause an abscess when injected subcutaneously. Of the various constituents of meconium which were tested by intratracheal injection, bile salts caused the most acute inflammatory reaction in the lungs, producing fatal pulmonary œdema with hæmorrhage and leucocytic emigration.

Human amniotic fluid was injected intratracheally in 14 animals. Of these, 5 which received 2 c.c. of fluid free from cells and solid material, were killed from 3 to 14 days later. Although some of this fluid was contaminated with bacteria, changes in the lungs were slight or absent. When a lesion was found, it consisted of slight collapse and was associated with perivascular lymphocytic infiltration.

In a group of 3 animals, cells from human amniotic fluid were injected. In one case these were suspended in amniotic fluid ; in the other two, they were washed free from amniotic fluid with saline, and were suspended in saline. In all three, sterility was obtained by autoclaving the material to be injected. The amount of fluid used ranged from 2 to 10 c.c., and the rabbits were killed after intervals of 3, 4 and 9 days. After 3 and 4 days there was some polymorphonuclear infiltration. In all of them the reaction, which was associated with collapse, showed many macrophages, some of which had coalesced to form foreign-body giant cells (fig. 7).

The remaining 6 animals each received 2 c.c. of thick amniotic deposit artificially stained with meconium and slightly contaminated with Gram-positive cocci and Gram-negative bacilli. The contamination was thought to have occurred during the collection of the fluid. In an animal killed 10 hours after such an injection there was collapse of alveoli with abundant polymorphonuclear infiltration. Keratinised squamous cells were recognised in the alveoli (fig. 8) and there was perivascular lymphocytic infiltration. In the animal killed after 5 days the changes were still marked but now the keratinised cells had disappeared and many giant cells were present (fig. 9). There were also signs of absorption by lymphatics, as indicated by cellular infiltration and dilatation of the sub-pleural lymphatics (fig. 10). The remaining animals were killed after 8, 11, 15 and 32 days. No keratinised cells could be found in the lungs, which showed gradual resolution of the inflammatory lesions, but even in the animals killed after 32 days small scattered areas of collapse with foreign-body giant cells could still be found on careful microscopic examination, and macrophages containing bile pigment were found in an area of lymphoid tissue.

DISCUSSION

The chief difficulty in interpreting these results is that observations upon the lungs of adult animals may not be applicable to the lungs of fœtal animals. In post-fœtal life, fluid of even the most bland nature, although it is rapidly absorbed from the lungs (Courtice and

Phipps, 1946-47), seems to cause collapse and inflammation in proportion to the amount of fluid introduced.   This is in accord with the findings of Miller *et al.* (1949) in dogs.   Though these workers reported that rabbits showed little inflammatory response to saline, the rabbit injected with saline in the present series showed distinct changes.

In the case of human amniotic fluid the cellular ·debris has an additional irritant effect and can induce a foreign-body˙ reaction. The injected cells cease to be recognisable in the lungs in the course of about 5 days.   The inflammatory changes generally are enhanced by meconium staining of the amniotic fluid.   The presence of perivascular round-cell infiltration and of dilated sub-pleural lymphatics suggests that lymphatic absorption is involved in the removal of the amniotic material from the lungs.   These lesions are usually not severe and resolve completely in a few weeks.   They produce no obvious illness in the animal.

The interest of these observations is in their relation to the lungs of the foetus *in utero*.   The foetal lung is certainly capable of an inflammatory response, as is proved by the presence of inflammatory areas in the lungs of some stillborn babies (MacGregor, 1939).   It might reasonably be presumed that, unless there is some marked difference between foetal and adult lungs, the inhalation of liquor amnii into the alveoli of the foetus would lead to a low-grade inflammatory response.   If such inflammation were not very widespread the baby might show no clinical evidence of the lesion, which would disappear within the first few days or weeks of life.   If such inflammation were very extensive, the baby would be liable to die at birth so that the pulmonary lesions could be found microscopically. A search has been made for foreign-body giant-cell reactions in the lungs of several hundred cases of stillbirth and neonatal death.   Such a reaction is very rare, and it is not mentioned by Johnson and Meyer (1925), Farber and Sweet (1931), Helwig (1933), Warwick (1934, 1937), MacGregor (1939), or Dick and Pund (1949).   Since chronic reactions are not found, it seems probable that amniotic fluid enters the lungs of the baby only when it is in distress.   The acute reactions sometimes found in the lungs may well have developed during the course of a protracted labour.   That the lungs of the newly born baby are capable of a foreign-body reaction is shown in fig. 11.   This presents a picture of multinucleated giant cells in the lung of an infant which survived for 4 days with many squamous cells in its alveoli.   The rarity of such a finding indicates that few babies which have inhaled amniotic fluid survive for any length of time, and it seems probable that a baby inhales before birth only when it is in difficulties.

SUMMARY

An inflammatory response occurs after the introduction of saline, rabbit amniotic fluid or human amniotic fluid into the lungs of adult

AMNIOTIC FLUID IN LUNGS

FIG. 1.—Film of deposit which settled from human amniotic fluid.   Gentian violet.   × 140.

FIG. 2.—Squamous cells in the lumen of a small bronchus in a stillborn infant.   Hæmatoxylin and eosin.   × 65.
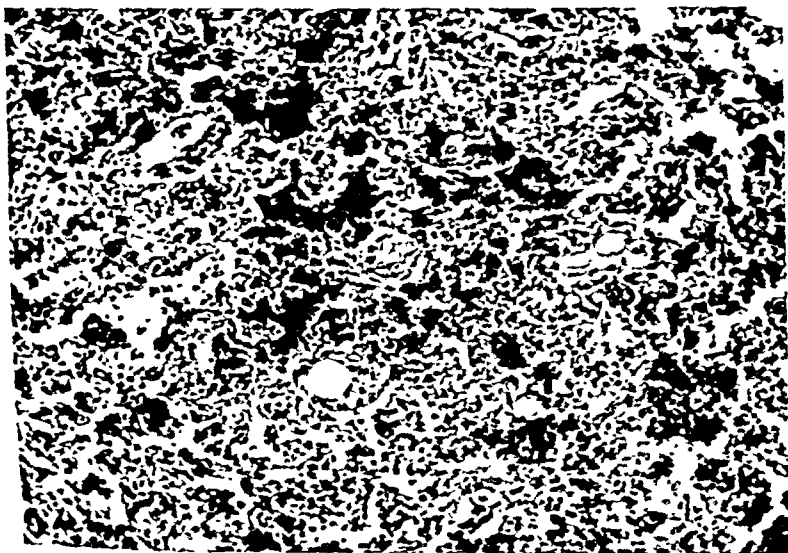
FIG. 3.—Collapse of lung following the introduction of the rabbit's own amniotic fluid.   H. and E.   × 170.

AMNIOTIC FLUID IN LUNGS
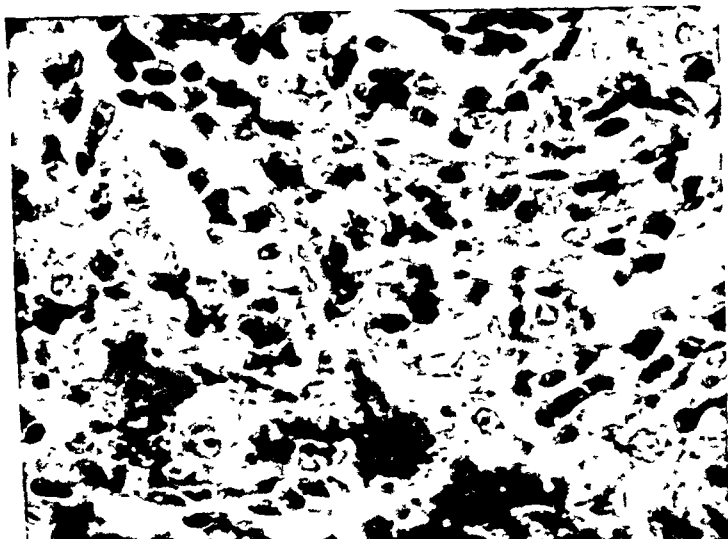
FIG. 4.—High-power view of same lung as in fig. 3.   H. and E.   × 630.

FIG. 5.—Collapse of lung following the introduction of normal saline.   H. and E.   × 155.
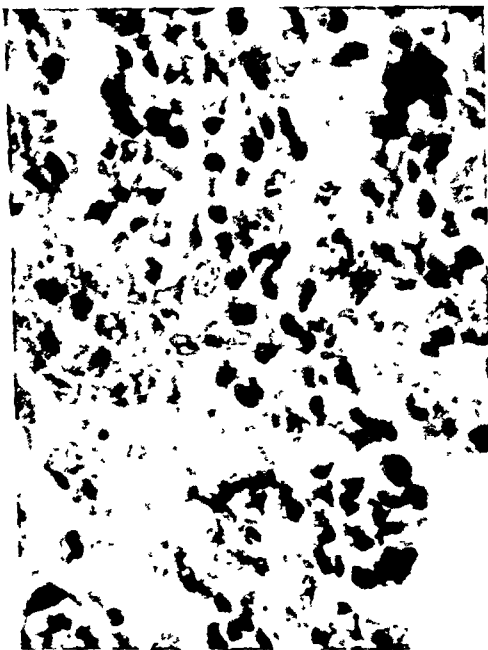
FIG 6.—High-power view of the same lung as in fig. 5.   H. and E.   × 630.

AMNIOTIC FLUID IN LUNGS
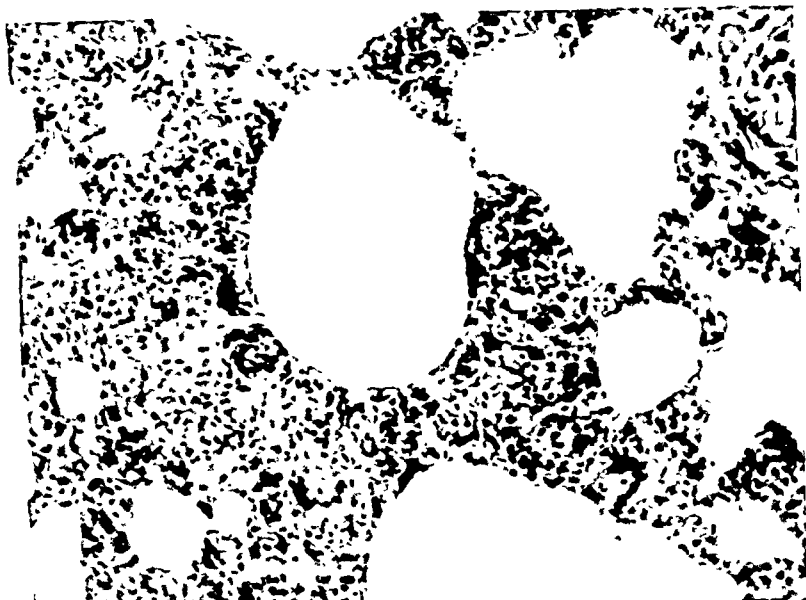
FIG. 7.—Foreign-body giant-cell reaction 9 days after the introduction of washed amniotic deposit.   H. and E.   ×170.
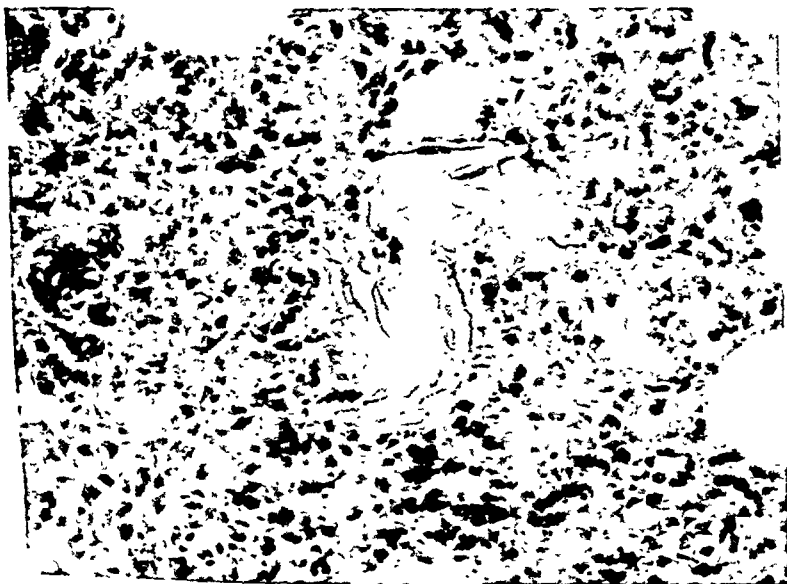
FIG. 8.—Keratinous squamous cells in a rabbit's lung 10 hours after the introduction of human amniotic fluid.   H. and E.   ×350.

AMNIOTIC FLUID IN LUNGS

FIG. 9.—Foreign body reaction 5 days after the introduction of human amniotic fluid into a rabbit's lung. H. and E.   ×170.

FIG. 10.—Collapse of lung and dilatation of subpleural lymphatics : same lung as in fig. 9. H. and E.   ×150.
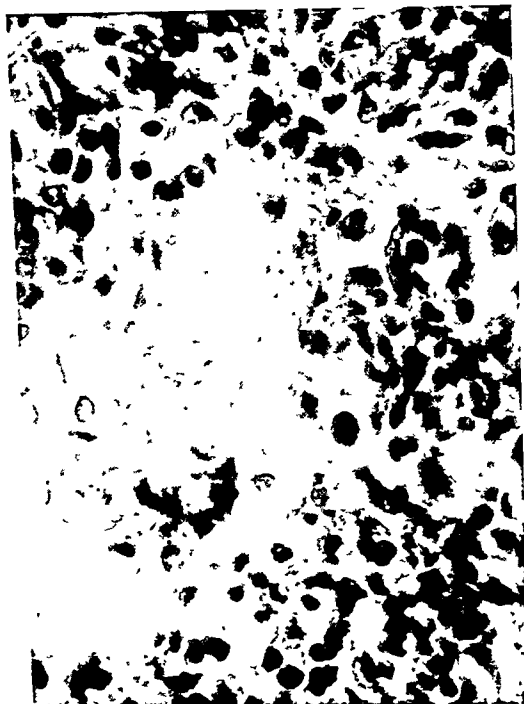
FIG. 11.—Foreign-body giant-cells in the lung of an infant which survived for 4 days with much amniotic debris in its lungs. H. and E.   ×585.

rabbits. If keratinised cells are present a foreign-body reaction results. It is concluded that it is abnormal for amniotic fluid to enter the lungs of the fœtus.

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A CASE OF INTRACEREBRAL XANTHOMATOSIS WITH PITUITARY INVOLVEMENT

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(PLATES CXXIX-CXXXI)

It is well known that the histological picture of xanthomatous lesions may vary considerably. The proportion of lipoid and granulomatous tissue, for instance, largely depends on the age of the lesions. When the lesions have been studied in their early stages the granulomatous reaction has been found but little or no lipoid deposit (Freund and Ripps, 1941). The granulomatous and more especially the fibroblastic reaction predominates too in very old lesions and occasionally tumour-like nodules are formed which are essentially fibrous in nature, with little remaining lipoid. The amount of eosinophil-cell infiltration also varies considerably and all grades of transition have been found between typical xanthomatous lesions in bones and so-called eosinophilic granulomata (Green and Farber, 1941).

Free cholesterol crystals are often liberated into the tissues in xanthomatous lesions, but from accounts in text-books and published articles, it would appear that this is of infrequent occurrence in the lesions of the Hand-Schüller-Christian syndrome. The following instance illustrates among other things the varied histological picture that may sometimes be encountered, even in a single case.

CASE REPORT

Clinical history

The patient, a male, was aged 17 years at the time of his death on 19.2.47. He had had nasal diphtheria at the age of 3½ years and measles and rubella as a baby. His father was killed during the war : his mother and three brothers and sisters are alive and well.

He was first seen at St Bartholomew's Hospital on 4.9.43, with one year's history of passing urine unconsciously at night and polyuria every three-quarters of an hour during the day. Polydipsia also had been present for one year. He had had frontal headaches at about weekly intervals for some years. These lasted about an hour and terminated after vomiting. On examination a slight lid lag was noted in both eyes. There was no exophthalmos.

Sept.-Dec. 1943. The patient was investigated and considered to be suffering from diabetes insipidus. He was treated with pitressin tannate

injections with improvement. The frequency of micturition, originally D/N = 20/30, improved to 3/0, but the headaches were unaffected.

On 11.9.44 he was readmitted for investigation by the neuro-surgical unit. The condition had relapsed somewhat. On examination he was thin, pale, intelligent and co-operative. Vision, hearing, concentration and memory were normal. The pulse, 62 per min., was normal. B.P. 110/70. Thyroid not palpable. Heart, lungs and abdomen normal. Urine, S.G. 1012. C.N.S., no gross changes. No swelling of discs. Slight weakness of 7th right cranial nerve. C.S.F. and X-ray of skull and pituitary fossa normal. Electro-encephalogram somewhat abnormal for age, but no focus of abnormality. Pneumo-encephalography showed no sign of intracranial tumour.

He was discharged in a stationary condition on 24.9.44 and during 1945 he was seen periodically as an out-patient. In April 1945 he complained of lassitude and poor appetite. Later this became severe ; he lost much weight and was thought to be developing Simmonds's Disease.

On 15.2.46 he was readmitted with these symptoms and the following investigations were performed, with the results shown :—Hæmoglobin 86 per cent. Plasma proteins : albumin 4·2 g., globulin 2·05 g. per 100 c.c. Blood urea 42 mg. per 100 c.c., Wassermann reaction of blood negative. Serum calcium 10 mg. per 100 c.c. Plasma chlorides 548 mg. per 100 c.c. Cholesterol estimations not performed.

X-ray examination of the chest, skull and sella turcica, often repeated, was always normal, of the elbows, wrists and hips, normal for the age. During this period of hospitalisation he was taken off pitressin, which led to considerable polyuria (average 3000 c.c. per day), but the specific gravity of some specimens was considered greater than one would expect in diabetes insipidus. Restricted fluids caused dehydration, hæmoconcentration and emaciation, without corresponding changes in the specific gravity of the urine. He was discharged on 21.4.46.

On 27.11.46 he was readmitted with failing vision in the left eye and drooping of the left eyelid. Ptosis was marked and there was weakness of left 3rd cranial nerve. He was blind in the left eye and there was a suggestion of proptosis. Both discs were pale. Oliguria was present, with headaches and drowsiness. The sella turcica was still normal.

On 29.1.47 he complained of failing vision in the right eye and was given 10 days' treatment with deep X-rays to the pituitary fossa, but the condition gradually deteriorated until his death on 19.2.47. He had lost weight steadily during the later stages of his illness—from 5 st. 1 lb. (32·2 kg.) in May 1946 to 4 st. 1 lb. (25·8 kg.) in January 1947.

Post-mortem findings (20.2.47)

The body was that of a grossly wasted adolescent male subject. The skin was dry and soft in texture ; the hair, fine and dry, pulled out easily. The face had a somewhat senile appearance. The mucous membranes were rather pale.

Serous sacs normal. *Tonsils* absent. *Thyroid* (15 g.). A relatively small left lobe : cut surface showed abundant colloid. *Lymph nodes* everywhere normal in size and appearance. *Alimentary canal* and *main air passages* normal.

Lungs (right, 435 g. ; left, 210 g.). Both were the seat of well marked atrophic emphysema. The right lower lobe showed patches of early bronchopneumonia and the finer bronchi and bronchioles exuded pus.

INTRACEREBRAL XANTHOMATOSIS

FIG. 1.—Slice of brain showing xanthomatous tissue infiltrating the region of the anterior commissure and lateral ventricles.

FIG. 2.—Slice of brain showing similar tissue in the caudate nuclei and lateral ventricles.

FIG. 3.—Lesion of brain showing the extent and arrangement of the reticulin fibres, the cholesterol clefts and the foreign body giant-cell reaction. Modified Foot's reticulin stain. × 120
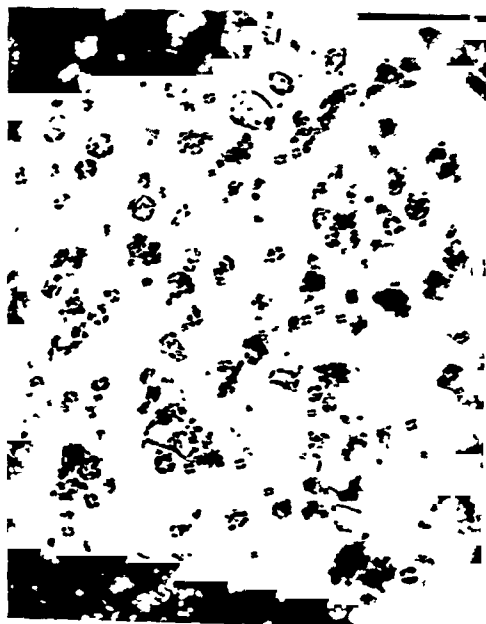
FIG. 4.—Brain Frozen section of xanthomatous tissue viewed with the polarising microscope. Fluid crystals of cholesterol esters are present in abundance, many of them giving the maltese-cross appearance. Scharlach R. × 300.

Heart (110 g.) and *liver* (660 g.), well marked brown atrophy. *Spleen* (40 g.), simple atrophy. *Adrenals* (5 g. together), normal apart from atrophy. *Kidneys* (right, 80 g. ; left, 75 g.). Capsules stripped easily, leaving surfaces which showed persistent fœtal lobulation. Cut surfaces normal. *Bladder, prostate* and *seminal vesicles* normal. *Testes* small in size and normal on section.

Bones. Right femur, sternum and vertebræ showed normal bone structure with no sign of xanthomatous deposits. The femoral marrow showed gelatinous degeneration. The skull presented a normal structure everywhere except on the dorsum sellæ, where the dura showed some localised granulations and was adherent to the bone. The orbits showed no sign of xanthomatous deposits.

Voluntary muscles everywhere appeared atrophied and dark brown in colour.

Brain (1250 g.). This showed some flattening of the convolutions and some surface œdema. Serial section revealed, in the region of the hypothalamus, an infiltration by yellowish tissue causing some local destruction of both grey and white matter. Anteriorly (fig. 1) this tissue had infiltrated beyond the anterior commissure, superiorly it partially infiltrated the thalamic nuclei and to a slighter extent the caudate and lentiform nuclei. The floor of the third and lateral ventricles (fig. 2), aqueduct and fourth ventricle was carpeted by this tissue. Inferiorly it permeated the infundibulum, the optic chiasma and the adjacent left optic nerve. The dural roof of the sella turcica was infiltrated and the pituitary gland partially destroyed by similar tissue. The choroid plexus, beneath the ependymal involvement, appeared normal, as did the brain stem and cerebellum.

Histology

The *heart, liver* and *quadriceps femoris muscle* present the appearances of brown atrophy. The *spleen* shows simple atrophy, the *kidneys* cloudy and autolytic change in the first convoluted tubules. The lower lobe of the right *lung* is the seat of marked hypostasis, much emphysema and a mild grade of bronchopneumonia. The *thyroid* acini are well filled with the resting type of colloid. There are no pathological changes. *Pancreas* and *prostate* normal. The *testes* show no gross architectural change, but there is a definite thickening of the basement membrane, very infrequent mitoses in the spermatocyte stages and mature spermatozoa are virtually absent. The *femur* shows no sign of xanthomatous tissue, but the fatty marrow is everywhere the seat of gelatinous degeneration.

Brain. The xanthomatous tissue has replaced much of the brain tissue in the region around and particularly below the aqueduct of Sylvius. It presents a granulomatous appearance, with a network of reticulin (fig. 3) and fine collagen fibrils spreading out from the blood vessels. Infiltrating cells are of many types—masses of small round cells,

probably microglial, larger pale eosinophilic cells of the " gemästete " type of glial phagocytes, eosinophil granulocytes, together with infrequent polymorphs and large mononuclear cells. Perivascular cuffing by lymphocytes is prominent at the periphery of the infiltrations, and in the contiguous brain tissue the nerve cells are in varying stages of degeneration.

Everywhere throughout the lesions are innumerable extracellular clefts where cholesterol crystals have been dissolved out of the section (fig. 5) ; these crystals have caused a striking foreign-body giant-cell reaction. In some sections aggregations of foamy cells are seen (fig. 6), but in general these are inconspicuous. Fat staining (Scharlach R) reveals abundant droplet deposits, both intra- and extracellular. Much of this fat is doubly refracting, and some of the droplets show the maltese-cross appearance of cholesterol esters in the fluid crystalline phase (fig. 4). Mallory's phosphotungstic acid-hæmatoxylin stain reveals a little glial reaction in some parts of the xanthomatous tissue. This, however, is not a prominent feature, though the vascular colla-genous granulation tissue is permeating into the brain tissue.

Sections of portions of the brain other than those seen to be affected naked eye, including cerebellum, pons and cerebral cortex, show no pathological change apart from prominent brown-atrophy pigmentation.

The *pituitary gland* (fig. 7) is infiltrated by similar granulation tissue, and by cells which include large mononuclears, lymphocytes, innumer-able eosinophils (fig. 8) and a number of large phagocytic cells containing fat vacuoles. Occasionally these are binucleate. Typical foamy cells, however, are virtually absent and cholesterol crystals are not seen. The infiltration has destroyed the whole of the pars posterior and a very small part of the adjacent pars anterior and has affected much of the dural capsule. The chromophobe, eosinophil and basophil cells of the pars anterior appear to be present in normal proportions.

The *optic chiasma* and *left optic nerve* are infiltrated by similar tissue, though the *right optic nerve* shows no infiltration.

Sphenoid bone. A small localised area in the dorsum sellæ shows the marrow in the medullary spaces to be replaced by xanthomatous tissue, which, however, appears to be less cellular than that in the sites already mentioned.

Chemical examination

Xanthomatous material was scraped from the brain substance after long formalin fixation and submitted to chemical analysis with the following results :—

Total cholesterol   .   .   .   . 15·2 per cent. of dry weight.
Free cholesterol   .   .   .   . 4·55 „   „   „   „   „
Cholesterol ester   .   .   .   . 10·65 „   „   „   „   „

Ratio of free cholesterol to cholesterol ester = 1 : 2·3

PLATE CXXX

INTRACEREBRAL XANTHOMATOSIS

FIG. 5 —Brain, showing xanthomatous tissue in region below aqueduct.   Cholesterol crystal clefts are present in abundance.   H. and E.   ×45.
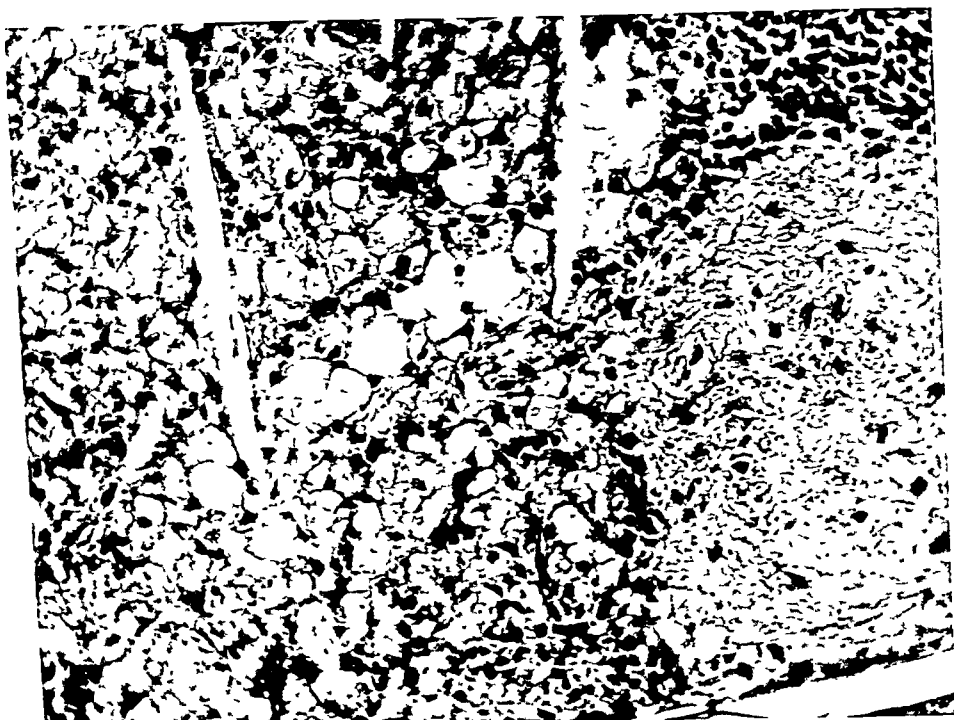
FIG 6 —Brain, showing one of the few foci of foamy-cell infiltration.
H. and E.   ×350.

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Ratio of free cholesterol to cholesterol ester = 1 : 2·3

DISCUSSION

This case presents a number of interesting and unusual features. The clinical signs of pituitary cachexia (anorexia, lassitude, low body temperature and steady loss of weight) in a patient already thought to have a pituitary lesion, supported by the post mortem findings of wasting, an appearance of senility, skin and hair changes and atrophy of organs including the endocrine glands, were at first sight difficult to fit in with the degree of involvement of the pituitary gland. However, cases of Simmonds's disease have been recorded with the responsible lesions situated in the pars posterior, the pituitary stalk or the brain itself. For instance, a cyst in the posterior lobe was described by Riecker and Curtis (1932), a gumma in the stalk by Jaffé (1922), and several tumours in the infundibular region of the brain have also been recorded. It seems, therefore, that the cachexia may have been produced by a pathological separation of the anterior pituitary from centres in the brain or by destruction of the centres themselves, or both. Depletion of chromophil cells, particularly eosinophils, in the pars anterior has been observed in cases of pituitary cachexia, but although extensive counts were not performed, the several histological sections of the gland revealed chromophil cells in approximately normal proportions (chromophobe cells 59 per cent., eosinophil cells 28 per cent., basophil cells 13 per cent. in a count of 1000 cells).

The classical triad of the Hand-Schuller-Christian syndrome can hardly be recognised in this case. True, towards the end of the disease a clinical note was made of a suggestion of proptosis in one eye, but at post-mortem, careful search failed to reveal any intra-orbital deposits or bony defect in the orbital region. Repeated X-ray examination of the skull and post-mortem examination failed to show any bony defects except for the minute area, already mentioned, in the extreme upper end of the dorsum sellae. This was intimately related to the dural infiltration around the pars posterior of the pituitary.

Diabetes insipidus therefore appears to be the only well-developed feature of the syndrome in this case, and although the specific gravity of the urine at times caused some doubt as to the diagnosis, nevertheless, according to Thannhauser (1940, p. 141), high figures have frequently been found in cases of diabetes insipidus and xanthoma disseminatum where bony changes were absent. The anatomical localisation of the lesions leaves little doubt that diabetes insipidus existed, and that the case is one variety of Hand-Schuller-Christian disease, which, as Henschen (1931) has pointed out, shows several clinical varieties.

The numerous eosinophils among the infiltrating cells, particularly well seen in the pituitary, where lipoid deposits were extremely scanty, are a feature which is occasionally seen in the xanthomatous deposits in Hand-Schuller-Christian disease. Eosinophilic granulomata without

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lipoid deposits are found of course most frequently in bones, but very occasionally other organs have been involved in an identical type of lesion in cases of clinically typical Hand-Schüller-Christian disease (Mallory, 1942). Routine examination was made only of the sternum, vertebræ, skull and femur in this case,. but in none of these bones were xanthomatous deposits seen, apart from the minute area in the sphenoid.

Van Bogaert et al. (1937) have described a very unusual case in which the total serum cholesterol was not increased and the chemical analysis of xanthomatous material from tendon swellings showed the extremely high free cholesterol : cholesterol ester ratio of 40 : 1 (Van Bogaert et al., p. 146), a reversal of the usual state in xanthomatous lesions. Cholesterol crystals were numerous in the lesions, and had provoked a marked foreign-body giant-cell reaction. Another feature of their case, unique in the literature, was involvement of the central nervous system sufficiently severe and widespread to cause definite neurological symptoms and signs. Similar lesions were found in the tendons and pleura. Van Bogaert and his colleagues (pp. 118-124) draw a distinction between their case and the few other recorded cases of intracerebral involvement by xanthomatous lesions (Chiari, 1933 ; Davison, 1933 ; Heine, 1934-35). Briefly these distinctions are :—

1. Absence of typical symptoms and signs associated with the Hand-Schüller-Christian syndrome.

2. Clinical signs of central nervous system involvement, confirmed by finding brain and cervical spinal cord lesions of unique distribution.

3. Deposition of innumerable cholesterol crystals with foreign-body giant-cell reaction.

4. Absence of granulomatous lesions, but macroglial reaction only, in the brain tissue proper.

5. Reversal of the usual free cholesterol : cholesterol ester ratio in the tendon lesions analysed.

Epstein and Lorenz (1937) have described the chemical and histological findings in this and a second similar case, and have classified the primary cholesterol lipoidoses into two groups according to the chemical constitution of the xanthomatous deposits.

Although the case of Van Bogaert et al. is unique in respect of its clinical manifestations and the anatomical distribution of the central nervous system lesions, nevertheless it would seem that the histological appearances are not so specific as is suggested and that such a classification as Epstein's is artificial, the differences being probably due mainly to the age of the lesions.

The distribution of the xanthomatous tissue in the brain of the present case differs considerably from that in the case of Van Bogaert et al. (p. 71). The histological feature of abundant extracellular crystalline cholesterol deposits, however, is obviously much the same in the two cases, although they differ in other respects. Eosinophils, abundant in· the present case, are not mentioned in Van Bogaert's

INTRACEREBRAL XANTHOMATOSIS
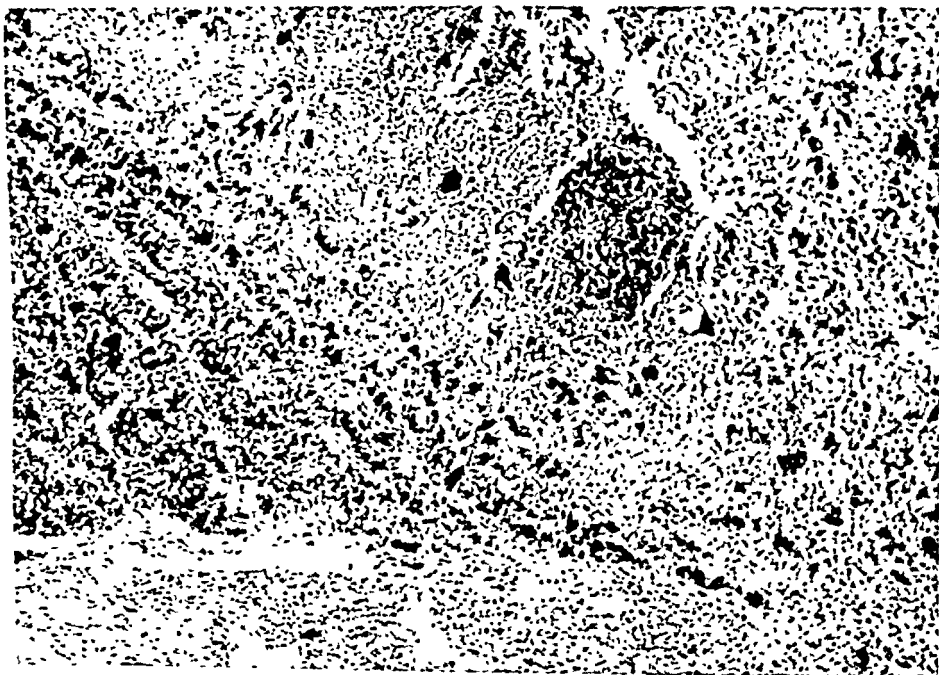
Fig. 7.—Pituitary gland showing xanthomatous granulation tissue infiltrating the capsule, the pars posterior, and part of the pars anterior. Hæmatoxylin and eosin. ×60.
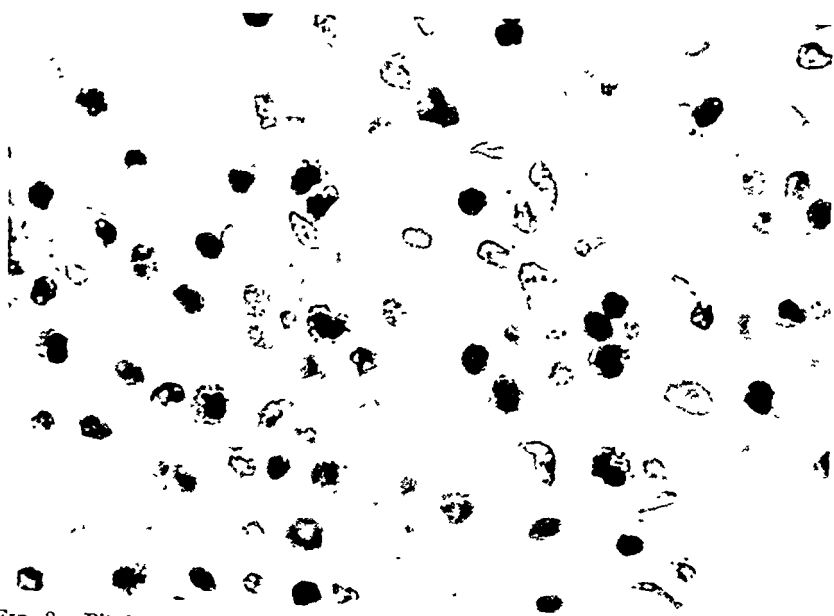
Fig. 8.—Pituitary. Granulation tissue containing numerous eosinophil granulocytes. H. and E. ×850.

case, nor did it show the marked gliosis of the surrounding brain. The histological appearances generally differ appreciably from most descriptions of the xanthomatous deposits of the Hand-Schüller-Christian disease, where foamy cells are usually abundant and cholesterol crystalline deposits are not stressed. The possibility that the cholesterol crystals were related merely to degenerated cerebral tissue was considered, but since the same crystalline deposits were found in other organs, *e.g.* the tendon and pleural lesions in Van Bogaert's case, the thymus in Merritt and Paige's case (1933), the dura in Rowland's case (1928), etc., it is felt that they are an integral part of the xanthomatous lesions in the present instance.

The chemical analysis is probably inaccurate owing to the possible inclusion of some normal brain tissue with the xanthomatous material examined, and to loss of cholesterol substances during formalin fixation, as pointed out by Epstein (Van Bogaert *et al.*, p. 150). Nevertheless, the total cholesterol content is still higher than in normal brain and is comparable with Epstein's total cholesterol values, while the ratio is in the usual region for xanthomatous tissue. In spite of the masses of cholesterol crystals in the histological lesions, however, the ratio does not compare with the ratio for Van Bogaert's tendon tissue.

It seems, therefore, that this case is of great interest in that it shows features which closely resemble both eosinophilic granuloma of bone and the Van Bogaert type of lesion, as well as many features which undoubtedly link it with the Hand-Schüller-Christian syndrome. Thus it helps to support the modern contention that eosinophilic granuloma and the Hand-Schüller-Christian syndrome are closely related, and seems also to link the Van Bogaert type of lesion, showing overwhelming predominance of free cholesterol deposits, with the more usual picture in the Hand-Schüller-Christian syndrome, where these are usually minimal or absent.

SUMMARY

The clinical and autopsy findings in an unusual case of the Hand-Schüller-Christian syndrome are described. The clinical features were those of diabetes insipidus followed by a Simmonds's type of cachexia. The patient, a male, was 17 years of age at the time of his death and the total duration of symptoms was 4½ years. Post-mortem examination revealed lipoid-containing granulomatous lesions in the brain, meninges and pituitary gland. Similarities are noted on the one hand to a case described by Van Bogaert, Scherer and Epstein in 1937, and on the other to eosinophilic granuloma.

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RED-CELL CHANGES IN BURNS AND ACUTE ANHYDRÆMIA

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The occurrence of anæmia after burns may be an important complication and many factors have been shown to play a part in its causation (Moore *et al.*, 1946 ; Cope, 1947). Various changes in the red corpuscles have been described in burns, in man as well as in experimental animals, due to thermal bombardment of the blood cells. These include hæmolysis with hæmoglobinæmia and hæmoglobinuria, crenation, fragmentation and budding, spherocytosis and microspherocytosis and increased fragility. Depressed bone-marrow function seems to occur in some of these cases and has been thought to contribute to and to maintain the anæmia, especially in its earlier stages. At the same time, acute anhydræmia resulting from excessive fluid loss at the site of the burn is known to play a dominant rôle in the pathology and symptomatology of the initial stages of burns.

Acute anhydræmia induced by the subcutaneous injection of hypertonic glucose solution has also been shown by Cameron *et al.* (1946) to give rise to anæmia lasting for a variable length of time from the third day onwards in goats and rabbits. These facts led us to investigate the effects of anhydræmia on the morphology and fragility of red blood cells and on the bone-marrow response, and also to evaluate the direct effects of heat on the red corpuscles.

METHODS

Healthy adult male rabbits of about 2-5 kg. body-weight were used throughout. Acute anhydræmia was produced by subcutaneous injection under light ether anæsthesia of 60 per cent. glucose solution (20 c.c. per kg.) according to the technique described by Cameron *et al.* (1946). Five per cent. glucose solution, which is approximately isotonic, was used for the control experiments. Samples of blood were taken from an ear vein before the injection and 1, 2, 4, 6, 8, 10 and 24 hours after the injection and heparinised. Hæmoglobin percentage, red-cell count and measurement of packed-cell volume were carried out according to the methods adopted by Cameron *et al.* (1945). Mean corpuscular volume was calculated. Mean corpuscular diameter was measured after the method of Price-Jones (1933), taking an average of 500 cells, and mean corpuscular average thickness was calculated. Mean corpuscular fragility was determined for each sample according to the method described by Dacie and Vaughan (1938) and any residual lysis and the presence of plasma hæmoglobin were looked for.

In another group of animals with anhydræmia induced in the same way, the bone-marrow response after repeated bleeding was investigated. Blood samples were taken immediately before and four hours after the injection of glucose. On each of the next two mornings 14 c.c. of blood per kg. body-weight were withdrawn after taking specimens for examination. Blood samples were then collected every morning for another 8-11 days. All these were examined for hæmoglobin percentage, red-cell count and reticulocyte content only. Control experiments after injection of 5 per cent. glucose were also · carried out.

An attempt was next made to ascertain whether the red blood cells are more susceptible to mechanical trauma in anhydræmic animals. Blood samples were withdrawn immediately before and 4, 8 and 24 hours after the injection of hypertonic glucose solution. The cells were traumatised by the following method. Test-tubes ($3'' \times 0.3''$) were half-filled with heparinised samples of blood and closed with rubber corks. These were placed vertically in a small wooden box with appropriate packing. The box was fixed to the axle of an electrically driven revolving machine. The test-tubes revolved at a rate of 50 revolutions per minute in a vertical plane, thus causing an up-and-down movement of the blood column. A similar procedure was carried out in a control animal injected with 5 per cent. glucose solution.

In a third group of animals 16-25 c.c. of blood were withdrawn into a conical flask, heparinised and heated for 2 minutes by placing the flask in a water-bath the temperature of which had been raised to 55° C. It was then cooled in a water-bath at 37° C. for 2 minutes and injected back into the animals through the ear veins. Hæmoglobin percentage and red-cell counts were determined before bleeding, 4-5 hours after injection and subsequently almost every morning for 4-13 days until the hæmoglobin returned to its original level. For control experiments the blood was kept in a water-bath at 37° C. for 4 minutes.

Five c.c. aliquots of blood from the ear vein were heparinised. One part was heated in a water-bath at 55° C. for 2 minutes and then kept at 37° C. for half-an-hour. The second part was heated at 85° C. for 5 seconds and at 37° C. for half-an-hour. The third part was kept in a water-bath at 37° C. for 30 minutes. This was followed in each case by an investigation of the changes in the red blood corpuscles by the methods described in the first group above.

In another set of experiments, 1 c.c. aliquots of heparinised blood were placed in water-baths at 37°, 45°, 55°, 65° and 75° C. respectively for 10 seconds. Leishman-stained thin smears were prepared from each of these samples and the percentage of cells showing microspherocytosis, crenation, budding and fragmentation was determined in counts of 500 cells. The percentage of hæmoglobin liberated in the plasma was also measured by comparing with standards prepared by hæmolysing the original packed cells in corresponding amounts of distilled water and making graded dilutions.

RESULTS

Effect of anhydræmia on the size and fragility of red corpuscles

There were 5 test animals and 5 controls. Tables I and II show these changes in a representative animal chosen respectively from the anhydræmic and the control series. It will be seen that the effect of anhydræmia in the test animals is manifested by a temporary rise in the hæmoglobin percentage, red-cell count and packed-cell volume, which, however, return to normal levels within 24 hours. These changes are absent in the controls. But the dimensions of the individual red blood corpuscles as indicated by the mean corpuscular volume,

mean corpuscular diameter, mean corpuscular average thickness and also the mean corpuscular fragility show no significant changes, nor is there any evidence of hæmolysis in the plasma.

TABLE I

Red-corpuscular changes in an anhydræmic animal (rabbit C, 2250 g.) injected subcutaneously with 20 c.c./kg. of 60 per cent. glucose

Blood	Time interval in hours after injection of glucose							
	Zero	1	2	4	6	8	10	24
Hæmoglobin (per cent. Haldane)	92	105	120	116	118	105	103	92
Red corpuscles (millions per c.mm.)	5·93	...	...	8·30	...	7·1	6·97	6·55
Packed-cell volume (per cent.) .	40·8	...	...	53·3	...	47·9	48·9	47·2
Mean corpuscular volume (cμ) .	68·8	...	...	64·2	...	67·5	70·2	72·2
Mean corpuscular diameter (μ) .	6·25	...	...	6·00	...	6·17	6·24	6·10
Mean corpuscular thickness (μ) .	2 24	...	...	2·70	...	2·25	2·30	2·46
Mean corpuscular fragility (per cent. NaCl)	0·395	...	...	...	...	0·415	...	0·395
Residual lysis (per cent.) . .	—	...	...	—	...	—	—	—
Plasma Hb.	—	...	...	—	...	—	—	—

TABLE II

Red corpuscular changes in a control animal (rabbit J, 2500 g.) injected subcutaneously with 20 c.c./kg. of 5 per cent. glucose

Blood	Time interval in hours after injection of glucose							
	Zero	1	2	4	6	8	10	24
Hæmoglobin (per cent. Haldane) .	82	...	86	84	84	84	83	84
Red corpuscles (millions per c.mm.)	6·12	..	. .	6 20	6·15	...	5·91	5·95
Packed-cell volume (per cent.) .	41·9	..	. .	40·3	42·6	...	38·5	41 8
Mean corpuscular volume (cμ) .	68·5	..	. .	65·0	69·3	...	65·1	70·2
Mean corpuscular diameter (μ) .	6·16	...		6·43	6·33	...	6·28	6·10
Mean corpuscular thickness (μ) .	2·30	..	. .	2·00	2·20	...	2·10	2·40
Mean corpuscular fragility (per cent. NaCl)	0·410	..	. .	...	0·430	...	...	0·430
Residual lysis . . .	—	...	...	—	—	...	—	—
Plasma Hb. . . .	—	...	...	—	—	..	—	—

Marrow response to bleeding in the anhydræmic animal

In this group there were three test animals and three controls. Tables III and IV show the effect on the reticulocyte response in the peripheral blood after two successive bleedings in a representative anhydræmic and normal animal respectively. No significant differences were observed between the test and control series with regard to the bone-marrow response as indicated by the reticulocyte counts. The red-cell count and the hæmoglobin percentage in each group fell after the bleeding but reverted to a normal level in 10-12 days. The

reticulocytes shot up and were maintained at about the same level in the two series before coming down to normal with the rise in the red-cell count.

TABLE III

Blood changes after bleeding an anhydræmic animal (rabbit 12, 3350 g.)

Time interval		Hb. (per cent.)	R.B.C. (millions per c.mm.)	Reticulocytes (per cent.)
15.12.47	Blood examination	94	6·34	2·45
	Injected subcutaneously with 20 c.c./kg. 60 per cent. glucose			
	After 4 hours	118	...	...
16.12.47	Blood examination	106	...	...
	Bled 14 c.c./kg. from ear vein			
17.12.47	Blood examination	62	3·47	1·71
	Bled 14 c.c./kg. from ear vein			
18.12.47	Blood examination	36	2·46	4·50
19.12.47	,, ,,	30	2·50	9·33
20.12.47	,, ,,	38	2·60	10·71
21.12.47	,, ,,	38	2·66	15·00
22.12.47	,, ,,	56	3·61	11·15
23.12.47	,, ,,	60	4·02	12·50
24.12.47	,, ,,	62	4·07	10·00
27.12.47	,, ,,	80	4·78	3·07
30.12.47	,, ,,	88	5·80	2·00

TABLE IV

Blood changes after bleeding a control animal (rabbit 15, 4150 g.)

Time interval		Hb. (per cent.)	R.B.C. (millions per c.mm.)	Reticulocytes (per cent.)
15.12.47	Blood examination	88	5·69	0·88
	Injected subcutaneously with 20 c.c./kg. 5 per cent. glucose			
	After 4 hours	94	...	...
16.12.47	Blood examination	86	...	...
	Bled 14 c.c./kg. from ear vein			
17.12.47	Blood examination	59	3·73	1·28
	Bled 14 c.c./kg. from ear vein			
18.12.47	Blood examination	42	2·85	2·86
19.12.47	,, ,,	45	2·86	5·00
20.12.47	,, ,,	52	3·65	14·47
21.12.47	,, ,,	56	3·61	11·57
22.12.47	,, ,,	60	4·06	9·69
23.12.47	,, ,,	70	4·43	10·00
24.12.47	,, - ,,	70	4·57	8·04
27.12.47	,, ,,	90	5·34	2·30

Effect of mechanical trauma on the red corpuscles of anhydræmic animals

There were two test animals and one control. There was no evidence of hæmolysis after mechanical trauma in any of the samples before or after the injection in either group.

Fate of heated blood after its replacement in the animal

There were five animals in each of the test and control series. There was no significant fall in the hæmoglobin percentage or red-cell

count in the controls (table VI), whereas in the test series, injected with heated blood, there was an appreciable fall in these constituents

TABLE V

Effect of bleeding and replacement of the blood after it had been heated to 55° C. for 2 minutes and cooled to 37° C. for 2 minutes (rabbit 4, 3080 g.)

Time interval	Hb (per cent)	R B C (millions per c mm)	Colour Index
Immediately before bleeding	88	6 39	0 69

25 c.c. blood removed, heparinised. heated and cooled as above and re-injected intravenously

After 4 hours	88	5 99	0·74
„ 1 day	86	6 05	0·71
„ 2 days	74	5 23	0·71
„ 4 „	68	4 84	0·70
„ 5 „	78	5·35	0·73
„ 6 „	82	5 62	0·73

TABLE VI

Effect of bleeding and replacement of the blood after it had been allowed to stand at 37° C. for 4 minutes (rabbit 9, 3550 g.)

Time interval	Hb (per cent)	R B C (millions per c mm)	Colour index
Immediately before bleeding	88	5 60	0 79

22 c.c. blood removed, heparinised, heated as above and re-injected intravenously

After 4 hours	84	5·62	0 75
„ 1 day	80	4·95	0·81
„ 3 „	82	5 50	0·75
„ 4 „	85	5 60	0 76

which, however, returned to the original level by about the 5th day (table V), except in one animal : this took 13 days to recover.

Effect of heat on the size of rabbit's red blood corpuscles

Table VII shows the changes undergone by red blood cells when exposed to different temperatures for varying periods of time. At 55° C. for two minutes, there was a diminution in the number of cells and the packed-cell volume, probably as a result of hæmolysis. The mean corpuscular volume did not show much change, whereas the mean corpuscular average thickness increased at the cost of the mean corpuscular diameter. These changes indicate an alteration in the contour of the cells, which is confirmed by the presence of many cells showing budding and also of fragmented cells. At the same time the mean corpuscular fragility was increased. It will also be seen

that exposure to 85° C. for five seconds did not cause a significant change in the number of cells, packed-cell volume, mean corpuscular volume, mean corpuscular diameter and mean corpuscular average

TABLE VII

Red-corpuscular changes after exposure to various temperatures for various lengths of time

	Temperature and length of exposure		
	37° C. for ½ hr.	55° C. for 2 minutes, then 37° C. for ½ hr.	85° C. for 5 seconds, then 37° C. for ½ hr.
Red corpuscles (millions per c.mm.)	5·75	5·08	5·90
Packed-cell volume (per cent.)	39·8	33	38·3
Mean corpuscular volume (cμ)	69·2	65·6	68·4
Mean corpuscular diameter (μ)	5·10	4·06	5·47
Mean corpuscular thickness (μ)	3·38	5·21	2·92
Percentage showing budding	0	12	4
Fragmentation	Nil	Present	Present
Mean corpuscular fragility	0·51	0·55	0·56

thickness, but there was definite evidence of budding and fragmentation, and of a rise in the mean corpuscular fragility. It would appear from the observations in this table that heat does affect the red blood cells directly and that the duration of the exposure to heat plays an important part.

Table VIII shows that when red blood cells are exposed to different temperatures for the same period of time (10 secs.) the morphological

TABLE VIII

Percentage incidence of red-corpuscular changes after exposure to various temperatures

	Temperature				
	37° C.	45° C.	55° C.	65° C.	75° C.
Normal	100	99	97·8	80	0
Small, deeply stained	0	0·5	1·4	8	18·4
Fragmented	0	0·5	0·8	6	16·6
Crenated	0	0	0	4	62·4
"Budding" cells	0	0	0	2	2·6

changes as well as hæmolysis vary directly with an increase in the temperature. These changes include microspherocytosis (change to smaller and more deeply stained cells), fragmentation, crenation and budding.

DISCUSSION

The results of these experiments clearly indicate that acute anhydræmia induced by the subcutaneous injection of hypertonic

glucose does not materially alter the morphology of the red blood corpuscles, nor does it increase their fragility or susceptibility to mechanical trauma. Moreover there is no evidence of a disturbance of bone-marrow function as indicated by the reticulocyte response to repeated bleeding. As has been shown by Cameron *et al.* (1946) this type of anhydræmia gives rise to many of the typical systemic features of burns. It is therefore reasonable to presume that anhydræmia, which is an inevitable accompaniment of the more severe burns, is not by itself responsible for any change in the morphology or fragility of red blood cells or for any depression of bone-marrow function in burns.

Nevertheless it has been shown that anhydræmia *per se* can cause anæmia in the experimental animal (Cameron *et al.*, 1946). It is tempting to assume that this anæmia in experimental anhydræmia has its counterpart in the false anæmia emphasised by Moore *et al.* after full-thickness burns of more than 20 per cent. of the body surface, an anæmia thought by these workers to be due to over-hydration of the blood. But Cameron *et al.* (1946) demonstrated that the anæmia in anhydræmic animals was unaccompanied by any dilution of the blood. Thus although anhydræmia definitely contributes to the anæmia of severe burns, the mechanism underlying the production of the anæmia still remains obscure.

The direct effects of heat on red blood corpuscles and its possible role in the causation of anæmia of burns now remain to be considered. It has been shown that heat causes varying degrees of hæmolysis, budding, fragmentation, crenation and microspherocytosis of red blood corpuscles, the number of these abnormal forms increasing with the temperature of exposure (table VIII), time too, playing an important part (table VII). Thus exposure to 85° C. for 5 seconds is less effective in causing these changes than exposure to 55° C. for two minutes. High temperatures also brought about frank hæmolysis and an increase in the mean corpuscular fragility.

Most of the morphological changes described in these experiments have already been found in blood heated *in vitro* (Schultze, 1865; von Lesser, 1880; Silbermann, 1890; Spiegler, 1896; Burkhardt, 1904-05; Isaacs *et al.*, 1924-25; and Shen and Ham, 1943). Spiegler, Isaacs *et al.*, and Shen and Ham have also demonstrated increased fragility of heated red corpuscles to isotonic saline, and Shen and Ham have shown such red cells to be more susceptible to trauma.

Moreover, when heated blood is injected back into the same or different animals of the same species, hæmoglobinæmia and hæmoglobinuria supervene and the morphologically altered cells are rapidly removed from the circulation (von Lesser; Shen and Ham). Shen and Ham have also demonstrated increased fragility of red corpuscles in the systemic circulation after about 1/5th of the total blood volume had been replaced by the same amount of heated blood.

Besides these in-vitro experiments, similar findings have been

recorded *in vivo*. Thus, similar morphological changes in red cells have been observed by various workers after experimental burns (Klebs, 1863 ; Wertheim, 1868 ; Ponfick, 1877 ; von Lesser, 1880 ; Pfeiffer, 1905). Recently, Moritz *et al.* (1947) have confirmed these findings and have also demonstrated increased fragility of the red blood cells of the burnt animal to isotonic saline. Hæmoglobinæmia or hæmoglobinuria or both have also been shown to occur after burns in experimental animals by Silbermann, von Lesser, Pfeiffer, and Moritz *et al.* At the same time absence of hæmoglobin or agglutinin in the serum or plasma has been demonstrated by Burkhardt. This observation suggests that antibody reaction was not responsible for the hæmolysis noted in the above experiments on burns.

Cases of burns in human beings, too, have been shown to develop exactly similar changes. Shen and Ham found spherocytes in the peripheral blood and an increase in the red-cell fragility in severely burnt patients. Brown (1946) showed fragmentation, microspherocytosis, increased mean corpuscular average thickness and increased mean corpuscular fragility in burns. Hæmoglobinæmia and hæmoglobinuria, either or both, have been frequently reported in such cases, especially those with severe burns involving a large area of body surface (Fraenkel, 1889 ; Tschmarke, 1896-97 ; Wilms, 1901 ; Shen and Ham, 1943 ; and Moore *et al.*, 1946). Shen and Ham failed to find any agglutinin or hæmolysin in the serum or plasma of such patients.

All this evidence points to the occurrence of gross lysis, increased fragility and susceptibility to trauma, as well as marked morphological changes in the red blood cells of burnt patients and animals. The in-vitro experiments show that heat alone is capable of bringing about such corpuscular changes and that the effects depend on both the temperature and the duration of exposure. As has been discussed above, the anhydræmia of severe burns plays no part in producing these effects on the red cells.

What is the significance of these red-cell changes for the anæmia that follows burns ? Moore *et al.* state that the cells involved in the initial hæmolysis do not exceed 10 per cent. of the total mass. Hardy and Soderstrom (1938) have shown that the blood-flow through the skin is 13 litres per hour per square metre of surface in the nude motionless subject, which amounts to 230 c.c. per minute per square metre. It can be calculated from these figures that, if all the corpuscles passing through one square metre of skin in one minute in full-thickness burns are involved and eventually destroyed, only 5 per cent. of the total blood in a 60 kg. man will be lost. Colebrook *et al.* (1944) concluded from their experiments that the volume of cells hæmolysed does not exceed 8 per cent. of the original volume. These facts indicate that a burn would necessarily have to be very extensive and would also require to involve the deeper structures in order to affect a large proportion of the circulating blood and to give rise to appreciable

anæmia. Colebrook *et al.* have also shown that the increased fragility of the red cells was confined only to a few hours after burns and was followed by subnormal fragility. In the present work, too, it has been found that in the majority of the animals the anæmia following the re-introduction of heated blood subsided by the fifth day. This suggests that the contribution of direct thermal changes in the corpuscles to the burn anæmia is transient.

Thus it is seen that these thermal effects do not explain the prolonged anæmia which follows the more severe burns. The other possible factors are blood loss through an open wound, infection. depressed marrow function as evidenced by a low reticulocyte count and impaired elaboration of iron in the circulating red cells (Moore *et al.*). Cope has also suggested fixation of iron at the vast inflammatory barrier in healing extensive burns. Vaughan and her co-workers (1946) are of the opinion that impaired liver function, manifested by plasma-protein disturbances, is responsible for a failure of hæmoglobin synthesis and thereby contributes to the anæmia of injuries. including burns. To these must be added the factor of anhydræmia demonstrated by Cameron *et al.* (1946), although the actual mechanism remains unexplained.

Summary and Conclusions

The role of acute anhydræmia and of direct heat on the morphology and fragility of the red blood corpuscles has been investigated. Direct heat produces severe changes in the form of hæmolysis, budding. fragmentation, crenation and microspherocytosis ; acute anhydræmia does not alter the corpuscles in any way. This is taken to indicate that such corpuscular changes as have been described in cases of burns are due to the direct thermal effect on the corpuscles, anhydræmia playing no part. Reasons are adduced to show that these corpuscular changes in cases of burns are not sufficient to account for the prolonged anæmia which may follow burning. Other possible factors are discussed, including the role of anhydræmia.

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DEFENSIVE MECHANISMS IN THE MEDIASTINUM, WITH SPECIAL REFERENCE TO THE MECHANICS OF PLEURAL ABSORPTION *

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(PLATES CXXXII-CXL)

THE infrequency of mediastinitis at post-mortems has often been noted by pathologists. Amongst 2808 autopsies performed at University College Hospital during the ten-year period 1921-30 there were only 22 cases of mediastinitis, as against 1558 which showed inflammatory changes in the lungs and pleuræ. It seems, then, that despite close contact with foci of sepsis, the mediastinal components escape infection, except in a very few cases.

This relative insusceptibility to infection, which has not been adequately explained, and previous observations (Karsner and Swanbeck, 1920-21; Seifert, 1928; Herrfarth, 1928; Higgins and Lemon, 1931) that the drainage of the pleural cavity takes place into the mediastinum, raises the question whether local cellular defence mechanisms exist in that structure. The present paper is chiefly concerned with the search for such mechanisms and the investigation of their mode of action.

REVIEW OF LITERATURE

Von Recklinghausen (1863) was aware of whitish " spots " composed of collections of cells traversed by a capillary network in the freshly exposed omentum and serous layers of the thoracic cavity in young rabbits. Ranvier (1874) called the omental spots " taches laiteuses " and in 1875 described similar patches about 0·5 to 3 mm. in diameter in the peritoneal surface of the diaphragm. Marchand (1901) suggested that these omental spots may be phagocytic in function, while Buxton and Torrey (1906), Higgins and Bain (1930), Webb (1931-32) and Thomas (1936) noted the active ingestion of particulate matter, such as bacteria, by the constituent cells. Maximow (1927b) found large numbers of amœboid elements in tissue cultures of omental transplants containing " taches laiteuses."

Very little attention appears to have been paid to the pleural " milk spots ". Maximow (1927a) mentioned them briefly as occurring in the mediastinal pleura of the dog, rabbit, guinea-pig, rat, cat and opossum. Kampmeier (1928), while investigating the fœtal thoracic duct, described as an incidental finding collections of cells with vascular networks immediately beneath areas of stratified meso-

thelium in the mediastinal pleura of the human newborn and resembling the
" milk spots " in the omentum.

A passing reference to the presence of a local protective mechanism in the
mediastinum is given in their textbook *The surgical diseases of the chest* by
Graham *et al.* (1935), who suggest that the mediastinum contains cells with
phagocytic properties.

Mixter (1941) made a detailed study of the Kampmeier's foci, as I shall
hereafter call them, and demonstrated their presence in the mediastinal pleura
of the rat, mouse, ground squirrel, grey squirrel, rabbit, cat, dog, mole and man.
He called them "macrophagal foci", because the constituent cells readily
ingested colloidal dyes and particulate matter such as trypan blue and India
ink when these were injected into the pleural cavity.

It would thus appear that both the peritoneum and pleura in mammals
harbour foci of macrophages. These may indeed be analogous to or even a
remnant of the phagocytic system in the cœlom of lower forms of life described
by Cameron (1932, 1934), especially the pericardial cells in caterpillars and
the cœlomic corpuscles in earthworms, which constitute a local protective
mechanism, ingesting with avidity a variety of particulate matter, including
micro-organisms.

Since it appeared likely that Kampmeier's foci may play a major role in
the defence mechanisms of the mediastinum, experiments were planned to
investigate in some detail their mode of response to various experimental
procedures.

MATERIAL AND METHODS

Various substances, including bacteria, were introduced into the pleural
cavity and their absorption studied by histological methods. A characteristic
and consistent path taken by particulates towards the very regions which
contain depots ideally suited to store irritant material was soon detected and
the motive forces governing the movement of particle suspensions within and
from the pleural cavity were studied. Irritant substances such as bacteria
were also introduced directly into the mediastinum, making use of avenues
by which infection usually reaches the mediastinum. In this way the evolution
of an experimentally produced mediastinitis and the means by which it
undergoes resolution could be followed.

In all, 157 adult white rats, 46 guinea-pigs and 19 rabbits were used: They
were fed with Rowett Institute (later M.R.C.) rat cubes, and they received an
ample supply of water.

The intrapleural injections were given in most cases into the right pleural
cavity through the fourth intercostal space under ether anæsthesia, using all
aseptic precautions. The thoracic viscera were fixed *in situ* by the injection of
10 per cent. formol-saline through the abdominal aorta immediately after the
animal was killed. The entire thorax with its contents was separated from the
rest of the body and placed in a vessel containing 10 per cent. formol-saline.
On the following day the thorax was opened and the soft structures, including
the mediastinal pleural folds, were removed and re-fixed in 10 per cent. formol-
saline. The bony wall of the thorax, including the parietal pleura and sternum,
was re-fixed in 10 per cent. formalin and decalcified later.

In serial experiments with particulates and bacteria, pieces of liver and
spleen were also fixed in 10 per cent. formol-saline. The thoracic viscera with
the pleural folds *in situ* were embedded in paraffin and transverse step sections
were stained with (1) Ehrlich's acid hæmatoxylin and eosin, (2) neutral red,
(3) Weigert's iron hæmatoxylin and Van Gieson, (4) Lendrum's (1947) stain for
reticulin, (5) eosin-Gram-Weigert stain for bacteria, (6) Ziehl-Neelsen's stain for
tubercle bacilli, (7) Gömöri's stain (1936) in the case of red-cell suspensions and
(8) Weigert's stain for fibrin. Mediastinal pleural spreads from rabbits, rats
and guinea-pigs were stained with Harris's hæmatoxylin.

RESULTS

The anatomy and histology of the pleura in relation to the mediastinum

The mediastinum of these experimental animals is composed of a cephalic portion with much cellular tissue and a caudal portion with very little cellular tissue (especially in the rat), separated from each other by the structures at the roots of the lungs. In the caudal
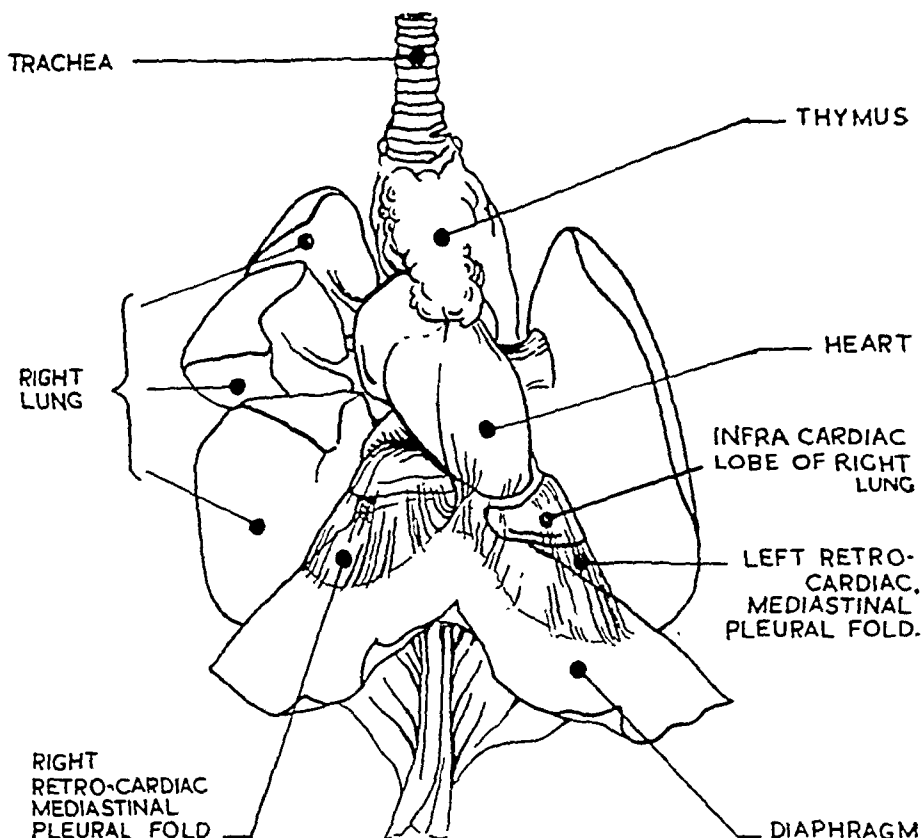
FIG. 1.—Ventral view of thoracic viscera of white rat, showing pleural reflections

portion are to be found certain pleural folds which will be shown to play a decisive role in absorption from the pleural cavity. The arrangement of the mediastinal pleura in the caudal part differs from that of man owing to (1) the position of the heart, which does not " sit " on the diaphragm as in man, (2) the presence of an additional lobe in the right lung.

The main point of interest is the presence of a pleural fold on each side of the midline which is reflected from the diaphragm *to* the pericardium and pulmonary roots (fig. 1). These folds will be referred to as the " retrocardiac mediastinal pleura " or " retrocardiac

pleura." They enclose a space bounded by the heart and pericardium cephalad and the diaphragm caudad. This may be termed the infracardiac space. Lobules of fat traversed by blood vessels are seen beneath these folds, as in the omentum, and adjacent to them are very small opalescent spots (Kampmeier's foci) which can be seen with a magnifying glass at intervals along the pleura. Similar spots, but not so numerous, occur in the folds between the œsophagus and aorta on the dorsal aspect of the animal.

The costal pleura is very firmly adherent to the intercostal muscles and ribs and the diaphragmatic pleura to the diaphragm. The cephalic portion of the mediastinal pleura is closely adherent to cellular tissue surrounding the trachea, œsophagus and great vessels. The retro-cardiac mediastinal pleural folds, on the other hand, in virtue of their attachment to perpetually moving structures like the heart, lungs and diaphragm, are subjected to maximum movement and stretching.

In pleural spreads, Kampmeier's foci appear as well-defined clusters of cells traversed by blood vessels (fig. 2). Injected spreads show clearly the intricate network of vascular channels within each focus, reminiscent of a glomerulus (figs. 2 and 5). These blood vessels are derived from fairly large trunks which traverse fatty lobules lying adjacent to some of the foci. By using the method of Feindel *et al.* (1947) it was shown that nerve fibres enter the pleura along with the blood vessels traversing the fat lobules. They almost encircle the foci and send small twigs into their substance. The mesothelial cells overlying each focus are morphologically different from those lining the rest of the pleura (fig. 3), being smaller and more variable in size (table).

TABLE

Measurement of mesothelial cells

	Mesothelial cells lining the Kampmeier foci	Mesothelial cells lining rest of pleura
Mean diameter . . .	16·4 μ	41·92 μ
Standard deviation . . .	6·8	9·5
Coefficient of variation . .	41·4 per cent.	2·2 per cent.

Sections made after impregnating the mesothelial cell boundaries with silver show that each Kampmeier's focus is situated immediately beneath the mesothelial cells, the more superficial elements actually abutting on the mesothelium (fig. 4). The cells of each focus, which are held together by a fine argyrophilic network (fig. 6), are mostly round with single vesicular nuclei, but with occasional multinucleated giant cells. The true macrophage nature of these cells is indicated by the readiness with which they ingest colloidal dyes and particulates. A characteristic feature is the presence of very large granular mast

FIG. 2. — Injected spread of retrocardiac pleura, showing several Kampmeier's foci with vascular networks. Harris's hæmatoxylin. ×60.

FIG. 3.—Spread of retrocardiac pleura showing part of a Kampmeier's focus. Mesothelial cell boundaries are outlined by silver. Note difference between mesothelium over focus and rest of pleura. Owing to the thickness of the spread, top left corner is out of focus, but the cell outlines can still be seen. Silver nitrate and Harris's hæmatoxylin. ×420.
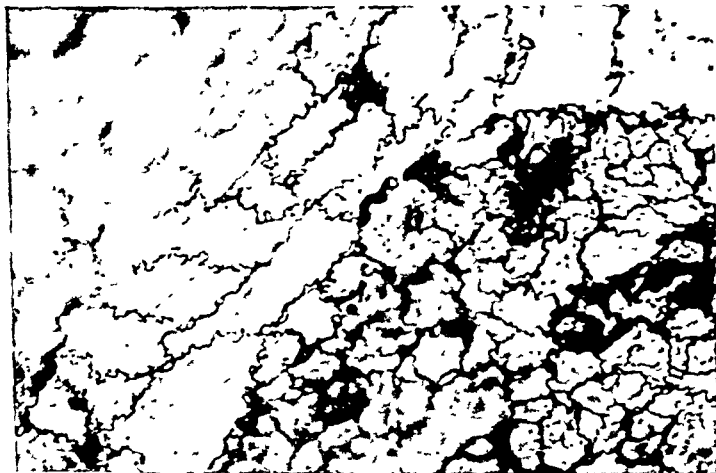
FIG. 4.—Section of silvered spread of retrocardiac pleura to show position of phagocytes in relation to the mesothelial lining. The silver impregnation can be seen between the mesothelial cells. Hæmatoxylin and eosin. ×850.

DEFENSIVE MECHANISMS IN THE MEDIASTINUM

FIG. 5.—Section of retrocardiac pleura after injection of blood vessels with India ink, showing the intricate vascular pattern in the Kampmeier's foci. The cellular appearance of the foci is due to previous intrapleural injection of trypan blue. Neutral red. × 65.
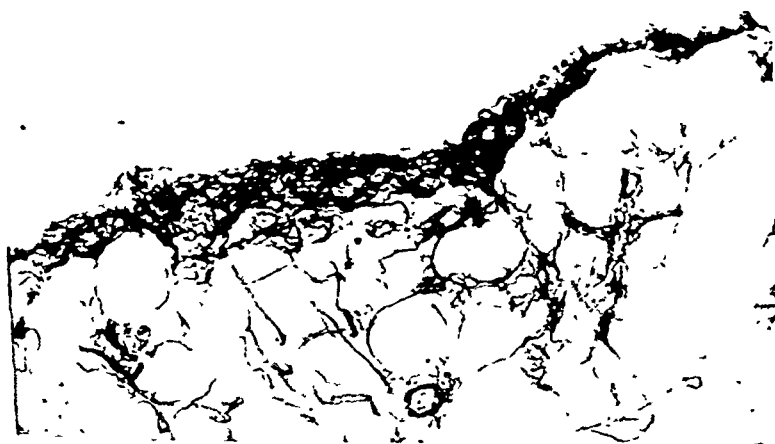
FIG. 6.—Argyrophil fibres at site of Kampmeier's focus. Silver impregnation. × 70.

PLATE CXXXIV

DEFENSIVE MECHANISMS IN THE MEDIASTINUM

FIG. 7.—Six hours after the introduction of India ink. Focal distribution in retrocardiac folds. Also foci in œsophageal-aortic fold. Neutral red. ×19.

DEFENSIVE MECHANISMS IN THE MEDIASTINUM

FIG. 8.—Ten hours after India ink. Focal distribution in retrocardiac folds.
One focus in œsophageal aortic fold. Neutral red. × 10.

DEFENSIVE MECHANISMS IN THE MEDIASTINUM

FIG. 8.—Ten hours after India ink. Focal distribution in retrocardiac folds. One focus in œsophageal-aortic fold. Neutral red. × 10.

cells. The histochemical method of Hale (1946) shows that the cells of the Kampmeier's foci are embedded in a matrix of mucin.

The absorption of particulates introduced into the pleural cavity

India ink, trypan blue, lithium carmine prepared according to Cappell's instructions (1929), suspensions of silica, rabbit's red corpuscles and vegetable oils were introduced into the pleural cavity of rats. In the case of India ink the animals were killed at the end of 2, 4, 6, 8, 10 and 24 hours, 2, 4, 7 and 14 days, and 1, 3 and 4 months, and a careful histological study was made in every case by cutting sections at different levels. A similar study on a smaller number of animals was made in the case of the other substances.

In all instances the particles find their way into the opposite pleural cavity through small fenestrations in the mediastinal pleura. They follow a characteristic and consistent course, spreading caudally towards the diaphragm and then cephalad along the mediastinal pleural folds towards the pulmonary roots. Most particles congregate over the retrocardiac folds : a few adhere to the œsophageal-aortic folds, the most dorsal part of the mediastinal pleura. Selective absorption at the Kampmeier's foci gives the appearance of a focal distribution of particles in these folds (figs. 7 and 8).

(*a*) *India ink.* Within 2-4 hours a fibrino-purulent reaction develops in the pleural cavity and large clumps of ink become attached to the foci by fibrinous strands. Particles commence to pass through the mesothelial lining in 6-8 hours and are immediately phagocyted by the macrophages of the foci. Polymorphonuclear leucocytes too, laden with particles, enter the foci simultaneously from the pleural cavity. Active phagocytosis goes on for the next few hours within the foci, the deeper cells of which also ingest ink particles (fig. 9). Increasing phagocytic activity is followed by proliferation of the macrophages, resulting in enlargement of the foci (fig. 10). By 24 hours most of the ink particles have passed into the foci and the polymorphonuclear leucocytes within them gradually diminish in number and finally disappear in two days. Only the large mononuclear cells now take part in phagocytosis. Fibrin disappears in 4 days and adjacent foci become coalescent on account of cell proliferation. A few ink-laden phagocytes commence to migrate between the underlying fat cells. By 7 days there is further cellular proliferation (fig. 11) and an increase in the reticular fibres in the foci becomes evident. From now onwards there is diminution in the cellularity of the foci, with an increase in the fibrous tissue, which commences to encapsulate masses of ink. Complete encapsulation by mature fibrous tissue and fixation of the ink masses in the form of nodules within the foci is seen in 3 months (fig. 12).

The costal, diaphragmatic and visceral pleura plays only a very small part in this absorptive process.

No free particles are seen at any stage in the lungs or mediastinal tissues. Ink granules are seen from time to time in the paratracheal glands but this is not a constant feature.

(b) *Trypan blue and lithium carmine* give similar results. They are absorbed at the Kampmeier's foci and phagocyted by the constituent macrophages.

(c) *Absorption of silica particles*, too, occurs at the Kampmeier's foci, which after a month show areas of necrosis. Free silica crystals are seen in the necrotic parts with polarised light, surrounded by fibroblasts and mononuclear phagocytes containing silica. At the end of 3 months fibrous silicotic nodules are found subpleurally in the position of the Kampmeier's foci.

(d) *Intrapleural injection of rabbit's red-cell suspensions* leads to proliferation of the macrophages in the foci at the end of 7 days, a large number of the phagocytes giving a positive prussian-blue reaction for hæmosiderin.

(e) *The introduction of vegetable oils* into the pleural cavity is followed by the appearance of large oil cysts in the Kampmeier's foci, surrounded by foamy macrophages (fig. 13).

The absorption of bacteria introduced into the pleural cavity

It was found that the introduction of 100 million bacteria suspended in 0·5 c.c. of normal saline could be tolerated and, at the same time, that those were numerically sufficient for the study of phagocytosis in sections. Injection of killed cultures made it possible to differentiate between phagocytosis on a large scale and multiplication of bacteria within a given focus.

(a) *Experiments with suspensions of killed staphylococci* (Staph. aureus). The animals were killed with ether at the end of half-an-hour and 1, 5, 24, 48 and 96 hours. Absorption of these organisms also takes place at the Kampmeier's foci in the retrocardiac fold. Phagocytosis by the macrophages of the foci commences within half-an-hour of their introduction and continues for an hour. After 5 hours this process is complicated by the appearance of polymorphonuclear leucocytes in the pleural cavity which ingest the cocci. Polymorphs laden with organisms now infiltrate the foci, where phagocytosis is continued. After this period proliferation of the macrophages of the foci commences and polymorphonuclear leucocytes and organisms diminish in number and disappear completely in 4 days. The foci become larger owing to the proliferation of macrophages. No organisms are seen in the mediastinal tissues or lymph glands and the escape of the lungs from invasion by organisms and from infection is a noteworthy feature.

(b) *Experiments with live staphylococcal suspensions.* The animals were killed at the end of 1, 2, 4, 6, 8, 12, 24 and 48 hours and 5, 7 and 14 days. As in the case of India ink, absorption takes place at the

DEFENSIVE MECHANISMS IN THE MEDIASTINUM
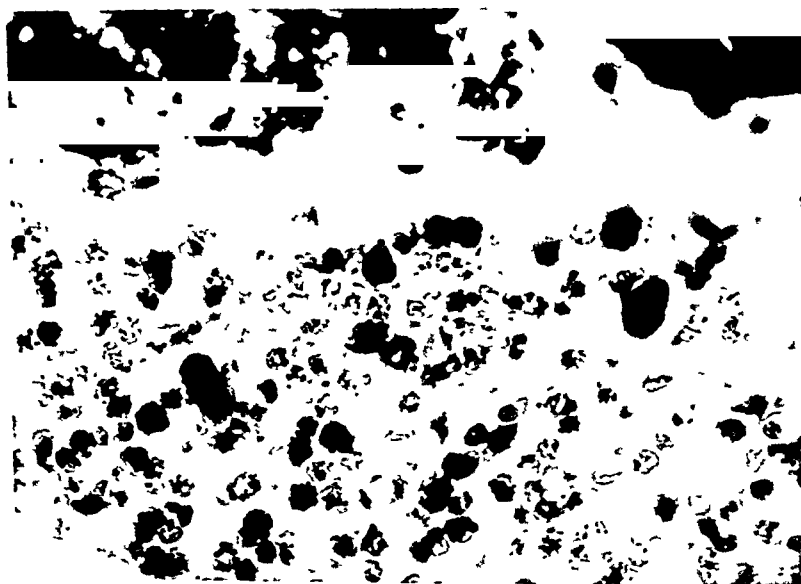
FIG. 9.—Twenty-four hours after India ink. Clump of ink on top. Phagocytes in Kampmeier's focus show intracellular granules. Neutral red. × 330.

FIG. 10 —Four days after India ink. Marked enlargement of foci, which are heavily stained. Neutral red. × 11.

Kampmeier's foci in the retrocardiac pleura (fig. 14), the constituent cells of the foci playing an active part in phagocytosis (fig. 15). The picture is complicated, however, by a more severe polymorphonuclear reaction. Organisms enter the foci and phagocytosis by fixed cells commences within an hour. A polymorphonuclear reaction in the pleural cavity is seen about the same time ; leucocytes ingest the cocci, adhere to the foci and subsequently pass through the pleura into the foci, carrying with them the ingested organisms. The efficiency of the clearing mechanism is such that within 6 hours there is a noticeable reduction in the number of organisms within poly-morphonuclear leucocytes in the pleural cavity and a remarkable increase of these cells laden with cocci in the Kampmeier's foci, the appearance being one of subpleural abscesses which increase in size in the next 2 hours.

By 24 hours there is active proliferation of the macrophages and greater phagocytic activity in the Kampmeier's foci, which now appear larger owing to congestion and œdema. After 48 hours only a few cocci are demonstrable within the macrophages of the enlarged foci. The deeper parts of the pleural folds often show abscesses which contain but few organisms. From now onwards the phagocytes in the foci continue to proliferate, while the organisms gradually diminish in number and ultimately disappear in 7 days.

The absence of organisms in and comparative freedom from involvement of the lungs and mediastinal tissues are noteworthy features. At no stage are organisms seen in lymphatic glands.

(c) *Experiments with live streptococcal suspensions* (Str. pyogenes, *group A Lancefield*). The animals were killed at the end of 1, 3, 6, 24 and 48 hours and 7 days. The results do not differ from those obtained with staphylococcal suspensions. Absorption takes place at identical sites and the bacteria are phagocyted by the macrophages of the Kampmeier's foci.

(d) *Avian tubercle bacilli and* (e) *Hofmann's bacillus*. The results are similar to those with staphylococcal and streptococcal suspensions.

(f) *Human tubercle bacilli in guinea-pigs*. The bacilli are absorbed from the pleural cavity at the same sites in 24 hours and their initial destination is the Kampmeier's foci, where an attempt is made by the phagocytes to destroy them (fig. 16). But the organisms multiply rapidly and spread in the mediastinal tissues, where a further attempt to localise them is made in 7 days by fibroblastic proliferation around bacterial concentrations. This, however, is unsuccessful, and in 14 days there is a diffuse spread of organisms in the tissue, with blood-stream dissemination.

(g) *Streptococci made virulent to rats by passage*. The animals died within 12 hours. The pleural cavities were filled with fibrino-purulent exudate and Kampmeier's foci were heavily infiltrated with leucocytes containing cocci. The mediastinal tissues also contained organisms.

*The forces which govern the movement of fluid suspensions
in the pleural cavity*

(a) *The effect of posture on absorption of particulates.* Posture or gravity does not cause a change in the course taken by particles within the pleural cavity. Although animals were maintained in different postures for 2 hours under Nembutal, the particles invariably moved towards the diaphragm caudally and thence cephalad along the retrocardiac folds towards the roots of the lungs. This experiment confirms the observations in the two previous groups, where it was noticed that the course followed by particulates was consistent, in spite of the different posture of the animals during long periods of observation.

(b) *The part played by certain moving structures in the thoracic cavity on the distribution of particles.* (i) *Cardiac movements.* The effect of the elimination of cardiac movements was studied by killing animals with ether, placing them on their backs and applying artificial respiration by means of an intratracheal canula connected to an electrically driven pump. The ink suspension was introduced into the right pleural cavity. Examination of the thorax in these animals after 2 hours of artificial respiration showed that, as long as the respiratory movements were maintained, the cessation of cardiac contractions did not cause an alteration in the course followed by the particles.

(ii) *Diaphragmatic movements.* Partial immobilisation of the diaphragm in guinea-pigs was effected by means of (a) unilateral phrenic avulsion (in the neck), (b) unilateral artificial pneumothorax, (c) pneumoperitoneum. In each case, particle distribution was carefully compared with normal controls breathing for the same length of time, due attention being paid to the extent of particle distribution (α) over the retrocardiac pleura in the experimental and control series, (β) over non-absorbing surfaces such as the visceral and costal pleura. It has been shown above that little or no absorption takes place from these sites.

With a *phrenic avulsion* on the left side and a normal right side, more particles were found on the right retrocardiac fold than on left and more particles were distributed over the costal pleura on left side. With a right-sided *artificial pneumothorax*, more particles were distributed on the left retrocardiac fold and over the costal pleura on the right side. With a *pneumoperitoneum*, very few particles were distributed over the retrocardiac folds. They were heavily concentrated over an area between the lungs dorsally and cephalad and a corresponding area on both sides of the vertebral column.

These experiments indicate that reduction of diaphragmatic movement brings about a corresponding reduction in the quantity of particulate matter reaching the area of absorption and that there is a greater tendency for this matter to remain over the

DEFENSIVE MECHANISMS IN THE MEDIASTINUM
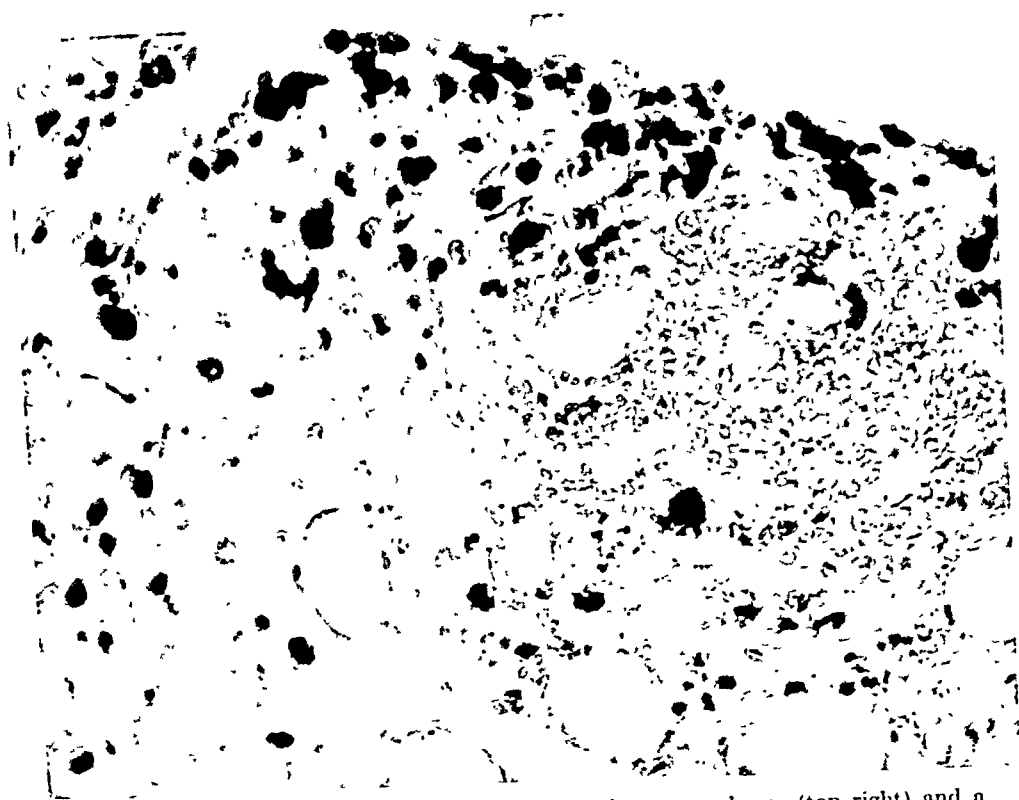
FIG. 11.—Seven days after India ink. Proliferating macrophages (top right) and a few ink-laden phagocytes migrating between fat cells. Neutral red. ×410.

FIG. 12.—Three months after India ink, showing a well encapsulated ink nodule. Van Gieson. ×90.
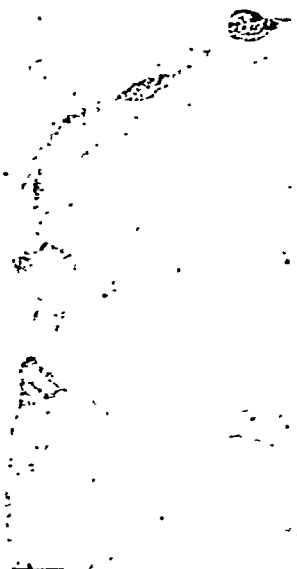
FIG. 14.—Twenty-four hours after the introduction of staphylococcal suspension. Focal distribution in Kampmeier's foci. Eosin-Gram-Weigert. ×25.

costal and visceral pleura from which little or no absorption takes place.

(c) *The mode of transmission of particulates through the mediastinal pleura.* (i) Two points needed clarification. Is transmission by phagocytosis, or can the particles be conveyed merely by the mechanical action of respiratory movements ? Recently killed rabbits were given artificial respiration followed by intrapleural injections of India ink suspension. After 1 hour's artificial respiration the mediastinal pleura was dissected out and examined histologically. Ink particles had passed through the mesothelial barrier and had even entered underlying lymphatic vessels. Phagocytes played no part in this process.

(ii) The exact mode of passage across the mesothelium was determined by careful serial section of the retrocardiac pleura 6 hours after the intrapleural injection of 0·2 c.c. of a 5 per cent. suspension of India ink. This clearly showed that ink particles passed both between the mesothelial cells and through their cytoplasm into the Kampmeier's foci.

(d) *The effect of the depth of respiratory movement on phagocytosis.* The depth of respiration was increased in a series of rats by partially obstructing the trachea. A control series of equal weight breathed normally. The same quantity of ink suspension was introduced into the pleural cavities. The intensity of phagocytosis in the two series was compared by studying sections of pleura after the animals had breathed for 3 hours. These showed that phagocytosis within the Kampmeier's foci was more effective in the animals with tracheal obstruction in which the depth of respiratory movement was increased. Thus dyspnœa definitely facilitates the passage of particulates through the pleura, and they are then more easily and more quickly ingested by the phagocytes of the foci.

(e) *The pathway for the absorption of fluid from the pleural cavity.* This was determined by the prussian-blue method advocated by Weed (1914-15) and Le Gros Clark (1929). The maximum deposition of prussian-blue granules occurred in the Kampmeier's foci, suggesting that these structures provide a natural exit for pleural fluids and for any particles in suspension.

(f) *Visualisation of the movement of radiopaque dyes within the pleural cavity.* Three dyes, Iodatol, Pyelosil and Viskiodone " 6," of different degrees of viscosity, were injected into the left pleural cavity of normal guinea-pigs and of those whose phrenic nerves had previously been avulsed. The course followed by these dyes was visualised on the fluorescent screen and exposures were made at intervals. The animals were nembutalised for this experiment, the dose being calculated according to body weight.

Summary of observations. The movement within the pleural cavity of a contrast medium depends on its viscosity and on the duration of the diaphragmatic paralysis. In the normal animal, contrast media of different degrees of viscosity follow the same path

as the particulate suspensions used in the previous experiments,
causing a constant and typical shadow in each case.   As expected,
the rate of movement varied inversely with the viscosity of the
medium, less viscous dyes moving faster.   That this path is determined
by diaphragmatic movement is shown by a deviation in its course in
cases of diaphragmatic paralysis.

(i) When the diaphragmatic movements were minimal, as in animals
with long-standing paralysis, there was hardly any movement of very
viscous dyes such as Iodatol.

(ii) When the same dye as in (i) was injected into animals with
paralysis of shorter duration, the dye slowly reached the diaphragm
but did not reach the retrocardiac folds.

(iii) When a medium of very low viscosity, *e.g.* Pyelosil, was used,
it was drawn to the unaffected side by the movements of the non-
paralysed hemidiaphragm more quickly than when the same medium
was injected into normal animals.

(iv) Simultaneous exposure of normal animals and of those with
paralysed hemidiaphragms after injecting a dye of moderate viscosity,
e.g. Viskiodone " 6," disclosed marked differences.   The normal
animals showed the typical shadow seen previously.   In animals
with diaphragmatic paralysis, there was delay in the movement of
the dye, the shadow at the injection site persisting for a longer period.
Most of it was drawn to the unaffected side and some of it remained
over the costal pleura, from which little or no absorption takes place.

These observations bear a close resemblance to the results obtained
when India ink suspensions are introduced into animals with
immobilised diaphragms.

(*g*) *The course followed by particulates in the dead animal.*   There
was no displacement of particles beyond some spread over a small
area around the injection site.   There was no tendency to gravitate
to dependent parts, although the animals were maintained in different
postures.

The fate of particulate matter introduced directly into
the mediastinal tissues

When the India ink suspension was introduced into the mediastinum
by puncturing the trachea inside the thorax, the greater portion
remained in the cephalic part.   Œsophageal puncture inside the thorax
enabled particles to be introduced into the caudal part.   Whichever
position the particles ultimately reached, two chief modes of spread
in the mediastinal tissues were observed :—

(i) A centrifugal spread in the tissue spaces, with a marked tendency
to a pleural drift.   The particles in this subpleural situation are
ingested by the phagocytes of the Kampmeier's foci, which permit
the escape of only a few particles into the pleural cavity.   Particles
are conveyed towards the pleura both within phagocytes and by free
passage in the tissue spaces.   That this pleural drift is a natural event

DEFENSIVE MECHANISMS IN THE MEDIASTINUM
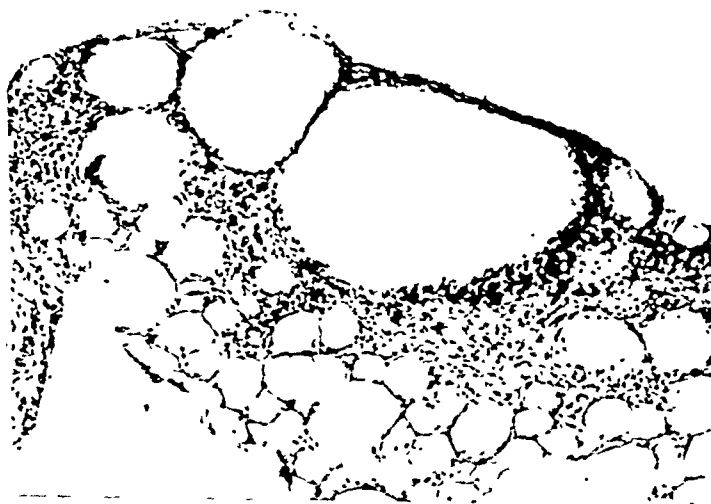
FIG. 13.—Formation of an oil cyst 8 weeks after the introduction of nut oil. H. and E. ×105.

FIG. 15.—Staphylococci within phagocytes in a Kampmeier's focus. Eosin-Gram-Weigert. ×750.
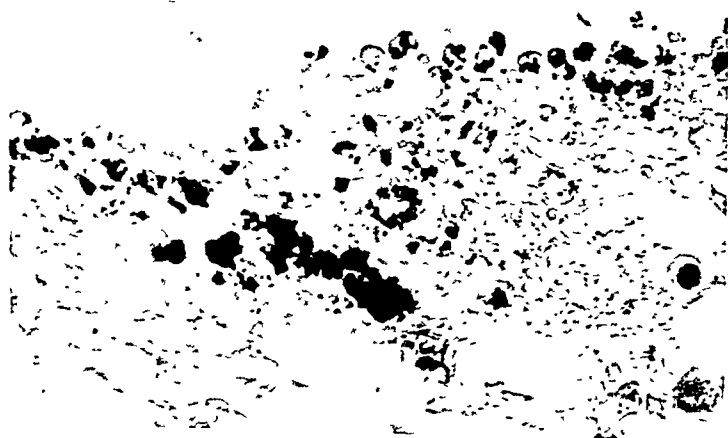
FIG. 16.—Guinea-pig. One day after the introduction of human tubercle bacilli. Kampmeier's focus showing marked cellularity and bacterial clumps. Ziehl-Neelsen. ×55.

is shown by the deposition of prussian-blue granules at identical sites (Weed : Le Gros Clark).

(ii) A caudal spread in the peri-œsophageal tissues. In this situation the particles are held up in the Kampmeier's foci of the retrocardiac and œsophageal-aortic folds.

There is no evidence that inert particles introduced into the mediastinum gain entry into the lung substance.

The evolution and resolution of an experimental mediastinitis

Half a c.c. of a *Staph. aureus* suspension (100 million) was introduced into the mediastinal tissues of rats by puncture of the trachea and œsophagus inside the thorax. The animals were killed with ether at intervals. This series provided a means of studying the mediastinal lesion at 24 hours and 2, 4, 7, 9, 10, 14 and 16 days.

In all but one case, it was found that the organisms had been introduced directly into the mediastinal tissues. In that case they had gained entry secondarily from a large œsophageal abscess. The spread in the mediastinal tissues was in all respects similar to that of India-ink particles (fig. 17). The acute stage of the mediastinitis commences in about 24 hours and is characterised by infiltration of the tissue spaces with polymorphonuclear cells and dissemination of organisms in the tissues (fig. 18). Pleural exudation begins almost simultaneously.

Attempts to overcome the infective process and to limit its spread are soon evident. The pleural drift tends to convey the bacteria into subpleural Kampmeier's foci, where many are held up and prevented from entering the pleural cavity. Those that do enter are quickly (in half-an-hour) phagocyted by the macrophages of the foci in the retrocardiac folds (fig. 19). Secondly, an attempt to localise the infection by abscess formation in the cephalic mediastinum is seen within 3 or 4 days (fig. 20), and thirdly, organisms tend to become localised in ulcers formed along the œsophagus.

The inflammation starts to subside in about 7 days. The bacteria disappear from the tissues, which now show foci of lymphocytic accumulation. The macrophages of the Kampmeier's foci, which had at first taken part in phagocytosis, continue to proliferate, resulting in marked cellularity of these foci. The organisms fail to reach the lungs, which, except for an area of patchy collapse following an œsophageal ulcer in one case, show no pathological change of any degree of severity.

The inflammation subsides in about 16 days. The pleural cavities are now free from exudate and the only evidence of previous injury to the mediastinal tissues are (1) patchy fibrosis, (2) focal lymphocytic accumulation, (3) marked cellularity of the Kampmeier's foci and (4) the accumulation of foamy cells in the retrocardiac folds.

The absorption of particulates from extra-thoracic structures
into the mediastinum

There does not appear to be any absorption from the soft tissues at the lower end of the sternum. Some slight absorption takes place from the neck. There is much absorption from the peritoneal cavity into the thorax, particles ultimately reaching the paratracheal glands, but the route followed does not appear to lie in the path of the Kampmeier's foci.

In-vitro experiments

Spreads of the retrocardiac pleura were made on slides with the mesothelial surfaces uppermost. The slides were immersed in trypan blue and potassium ferrocyanide solutions (Weed ; Le Gros Clark). Trypan blue and prussian-blue granules collected over the Kampmeier's foci, the rest of the spreads showing only a few granules.

DISCUSSION

An attempt has been made in this study to define a local cellular defence mechanism in the mediastinum. It has been shown that particulate matter introduced into the pleural cavity passes through definite zones in the mediastinal pleura. These points of exit are guarded by collections of macrophages (Kampmeier's foci) which lie immediately beneath the pleural mesothelium (fig. 4). This system acts as a very efficient protective mechanism, since particulates such as India ink, bacteria, red blood cells, oil globules, silica and colloidal dyes such as trypan blue are very quickly ingested by the sub-mesothelial phagocytes. Although the pleura actively participates in absorption (Starling and Tubby, 1894 ; Karsner and Swanbeck, 1920-21 ; Corper, 1926 ; Higgins and Lemon, 1931), the possible ill effects of such absorption are minimised by these barriers, which prevent the dissemination of irritants in the mediastinal tissues, and by the blood stream. Clinical experience of mediastinitis is in keeping with the conclusions reached from animal experiments. It has been shown that out of 564 infections of the pleural cavity there were only 22 cases of mediastinitis, and in 66 cases of mediastinitis described by Neuhof (1936-37) the infection only rarely came from the lungs or pleura. Structures morphologically identical with Kampmeier's foci in animals have been demonstrated in the human mediastinal pleura by Kampmeier (1928), Mixter (1941) and myself. The presence of these structures and the efficiency with which they perform the function of phagocytosis in animals may partly explain why the mediastinum is so resistant to infection from the pleural cavity.

Graham's (1921) experiments clearly demonstrated that fluid rapidly collects in the pleural cavity when the lung becomes œdematous from any cause. Brock and Blair (1931-32) confirmed this finding and also showed that a broth culture of Streptococcus introduced into the

FIG. 17.—Centrifugal spread of staphylococci in mediastinal tissues and collection in subpleural foci.   Eosin-Gram-Weigert.   ×13.

FIG. 18.—Experimental mediastinitis.   Diffuse inflammatory-cell infiltration of mediastinal tissues.   H. and E.   ×65.

DEFENSIVE MECHANISMS IN THE MEDIASTINUM

FIG. 19.—Organisms in Kampmeier's foci. No organisms over pleura between foci. Eosin Gram-Weigert. × 90.
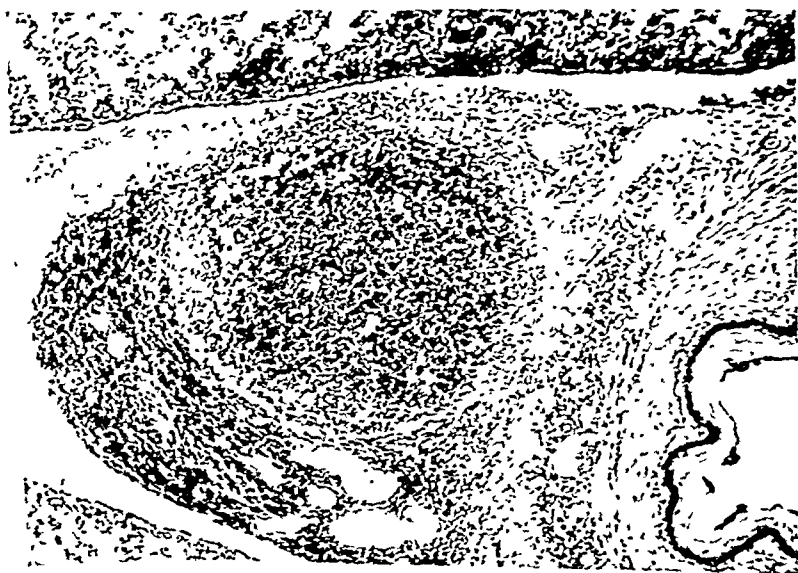
FIG. 20.—Abscess in mediastinal tissues. Œsophagus to right.
H. and E. × 65.

lungs through the bronchi passed into the pleural cavity almost immediately. This experimental evidence suggests that in pulmonary inflammations large quantities of bacteria-laden fluid may form in the pleural cavity. If this is a common occurrence, absorption by way of the Kampmeier's foci with the associated phagocytosis assumes great importance in preventing a mediastinitis or septicæmia. With sub-clinical infections of the lungs, too, it is possible that small fluid collections containing micro-organisms may accumulate in the pleural cavity from time to time without arousing suspicion. Because of the efficiency of these foci the pleural cavity may be rendered sterile and the mediastinal tissues and blood stream may be kept free from infection.

The activity of the Kampmeier's foci is intimately connected with the direction of movement of intrapleural fluids, which in turn is dependent on respiration, and it has been shown that the motive force is provided by the contractions of the diaphragm. During diaphragmatic descent in inspiration, a strong suction effect is exerted which causes the intrapleural contents to be drawn towards the diaphragm from the potential space between lung and chest wall. The expiratory act, being passive, is not of sufficient force to cause the fluid to be driven back to its original position. Therefore it escapes into the retrocardiac area between the lungs and retrocardiac mediastinal pleura, which, by bulging towards the infracardiac space, affords sufficient room for the accommodation of fluid (see fig. 1). This latter movement is also aided by cardiac systole, which, however, is not indispensable, since artificial respiration in the absence of cardiac contractions does not alter the direction of flow. The direction of movement of pleural fluid is thus towards these phagocytic collections.

The movement towards the absorptive zones is abolished by diaphragmatic paralysis, the result being that particles suspended in the fluid remain on sites from which hardly any absorption takes place. Bettman (1925) also observed that absorption of India ink was delayed in dogs with artificial pneumothorax and suggested that the physiology of the pleural space was so altered by the pneumo-thorax that the pleura was no longer able to dispose of the ink. The real explanation of this disability is that impairment of diaphragmatic movement consequent on the pneumothorax, together with the production of an air lock interrupting the continuity of the pleural fluid, results in a failure to convey the particles to the absorbing surface ; hence they remain in the pleural cavity. The diaphragm is therefore as important in pleural as in peritoneal absorption (MacCallum, 1903 ; Bolton, 1921 ; Cunningham, 1922b ; Higgins et al., 1930).

Absorption is preceded by the attachment of particulate clumps to the mesothelium over the Kampmeier's foci, giving rise to the appearance of a focal distribution along the mediastinal folds (figs. 7, 8 and 14). The penetration of particles occurs only at these foci,

which serve as natural pathways and as a means of exit for pleural fluids (*vide* experiments with potassium ferrocyanide solution). The transmission of particles through the pleural membrane is effected both by the mechanical act of respiration and carriage within phagocytes, the former operating in the early stages, the latter in the later stages of absorption. In their passage through the pleura, particles pass both between the mesothelial cells and through their living cytoplasm. These processes are essentially similar to those described by Cunningham (1922*a* and *c*) in the case of peritoneal absorption.

The explanation of these modes of passage through selective areas in the mediastinal pleura is to be found in the structure of the mesothelium lining the Kampmeier's foci, which is distinctly different from the rest of the pleural mesothelium (fig. 3). The smaller size of the mesothelial cells over the foci and the consequent increase in the total amount of intercellular space combined with the property of " stickiness " express the admirable adaptation of the Kampmeier's foci to the function of absorption. Although Heger (1904) and Buxton and Torrey (1906) noticed this property of stickiness in omental " milk spots," no sticky substance has so far been demonstrated. I have shown the presence of mucin in these foci, which no doubt assists in the attachment of bacteria to these phagocytic systems. When trapped in this manner they are more easily attacked by the macrophages—a phenomenon not unlike that of the " surface phagocytosis " described by Wood *et al.* (1946).

No absorption appears to take place through the costal, visceral or diaphragmatic pleura, as pleural fluid is driven towards the retrocardiac mediastinal pleura. Transmission of particles occurs 'through this membrane as it continually stretches and relaxes like a concertina on account of its attachments to vigorously moving structures such as the heart, lungs and diaphragm. Such stretching causes a separation of the mesothelial elements through which particles and fluid gain easy access to the subpleural tissues. According to Field and Drinker (1931), mechanical forces, especially stretching, aid the transmission of material through endothelial-lined structures.

The reactions of the Kampmeier's foci after the entry of particles depend on their nature, but the end result is similar in all cases, namely localisation of the irritant and prevention of its spread. The macrophages proliferate following phagocytic activity—a characteristic feature of cells belonging to the reticulo-endothelial system. Proliferation results in a coalescence of adjacent foci. With India ink there is ultimately complete encapsulation of the ink masses by mature fibrous tissue. The picture resembles that described by Webb in the omental " milk spots," the retrocardiac folds thus acting, in the pleural cavity, as the analogue of the omentum. Indeed, the structure of the Kampmeier's foci and their vascular arrangements closely resemble the injected rat's omentum as described by Simer

(1934). In the case of bacterial suspensions conditions are somewhat different on account of violent inflammatory reactions and polymorphonuclear exudation, but in general the sequelæ are similar. The introduction of silica suspensions and vegetable oils into the pleural cavity yielded similar results, while the intrapleural injection of red-cell suspensions was followed by the formation of hæmosiderin exclusively within the ingesting phagocytes in these foci. The absence of free particles in the mediastinal tissues was demonstrable evidence of the efficiency with which these foci acted as barriers in the pleura.

The intensity of phagocytosis in the Kampmeier's foci is influenced by the depth of respiratory movement. Thus although deep breathing or dyspnœa enhances absorption from the pleural cavity (Brock, 1933-34), any particles so absorbed are more effectively phagocyted by the macrophages, in reality an example of homeostasis (Cannon, 1932).

In the study of experimental mediastinitis it was observed that particles, both India ink and bacteria, when introduced directly into the mediastinal tissues follow a characteristic course in the tissue spaces, moving away from the midline structures towards the pleura— a centrifugal spread with a pleural drift (fig. 17). This acts as a safety device, causing irritants to leave vital thoracic structures and to be efficiently dealt with by phagocytes of the Kampmeier's foci. There is also a caudal spread towards the base of the mediastinum, where they reach the retrocardiac pleura with consequent exposure to the action of the foci in this situation. Increased negative pressure at the base of the mediastinum (Meltzer, 1892 ; Prinzmetal and Kountz, 1935) causes particle suspensions to be driven to the caudal part. These two modes of spread are mainly instrumental in preventing gross damage to the lungs and pleura. Another protective device which prevents dissemination in mediastinal tissues is the formation of localised abscesses (fig. 20).

The Kampmeier's foci, by virtue of their strategic position in the mediastinum, act as barriers to the spread of infection either from the pleural cavity or from the mediastinal tissues. They constitute the first line of defence in the mediastinum.

SUMMARY

The mediastinal pleura of animals contains macrophage collections, called Kampmeier's foci, which exist as definite structural units. On account of the avidity with which they ingest particulate matter, including bacteria, these foci may be regarded as a part of the reticulo-endothelial system. Because of structural differences of the mesothelium over these foci and their property of stickiness, they serve as exits for pleural contents. Thus irritants leaving the pleural sacs pass straight into these phagocytic collections, where they are held up and their further progress is retarded.

Movement towards the Kampmeier's foci is due to the diaphragmatic contractions and the anatomical arrangement of the mediastinum. Absorption is definitely delayed when the diaphragm is immobilised by phrenic avulsion, artificial pneumothorax or pneumoperitoneum.

The transmission of particulate matter through the mediastinal pleura is effected in the early stages by the mechanical action of respiration and in the later stages by carriage within phagocytes.

The Kampmeier's foci act as the first line of defence in the mediastinum, ingesting most particles and bacteria and fixing them in the form of abscesses in the latter case and as encapsulated nodules in the former. The retrocardiac pleura may therefore be considered as the pleural homologue of the omentum.

When experimental mediastinitis is produced by the introduction of particulates, the particles spread centrifugally towards the pleura—pleural drift—and caudally. Irritants thus move away from vital mediastinal structures and reach the Kampmeier's foci, which in this case protect the pleura.

Owing to their strategic position in the pathway of absorption of irritants both from the pleural cavity and the mediastinal tissues, their property of stickiness and their close proximity to the pleural sac, these foci form a very effective defence mechanism.

Structures resembling the Kampmeier's foci of the lower animals have been found in the human mediastinal pleura.

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SPLENIC LESIONS IN PERIARTERITIS NODOSA

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(PLATES CXLI-CXLIII)

THE histological diagnosis of periarteritis nodosa usually presents no difficulty, especially in the acute phase. Descriptions of recorded cases show, however, that the appearances may vary considerably, depending partly on the age of the lesion, partly on the size of the affected vessel and to some extent on the organ involved. Furthermore, Davson, Ball and Platt (1948) have already emphasised that, in the kidney, changes may occur in the parenchyma which are not immediately recognisable as characteristic lesions of periarteritis nodosa.

That the disease frequently involves the spleen is attested by the many cases reported in the literature and the estimated incidence has ranged between 14 per cent. (Gruber, 1925) and 31 per cent. (Harris *et al.*, 1939). Apart from occasional individual case reports such as those of Fishberg (1927), Gohrbandt (1927), Krahulik *et al.* (1935), Wegener (1939), Banowitch *et al.* (1942), and especially Klinger (1931), histological accounts of splenic involvement have been scanty, and no systematic histological study of the lesions in the spleen in a substantial series of cases of periarteritis nodosa has as yet been reported.

The purpose of this paper, therefore, is to describe the pathological changes found in the spleen in 16 cases of periarteritis nodosa, to indicate the range of appearances that may be encountered and to emphasise the occurrence of lesions that would not, in themselves, immediately suggest the diagnosis of periarteritis nodosa.

MATERIAL AND METHODS

Splenic tissue suitable for histological study was available in 17 out of 23 cases of periarteritis nodosa coming to autopsy between 1934 and 1948. One or more blocks of spleen were cut in paraffin. Hæmalum and eosin, Weigert's elastic-Van Gieson combination, Gömöri's reticulin stain and the prussian-blue method were used in all cases. When necessary, sections were stained also by Gram's method, Ziehl-Neelsen's method and Weigert's fibrin stain. In some cases large numbers of serial sections were prepared.

The diagnosis of periarteritis nodosa in these cases rests on the histological demonstration of the typical lesions in one or more organs and a clinical history

compatible with or definitely suggestive of this diagnosis. Cases classed on clinical or other grounds as disseminated lupus and the Libman-Sachs syndrome or scleroderma, although they may show similar vascular lesions, have been excluded.

Summaries of the clinical and pathological findings are presented in the appendix. Cases marked with an asterisk have already been reported with special reference to the renal lesions (Davson *et al.*).

Control series. The control group consisted of material from 400 cases coming to autopsy at the Manchester Royal Infirmary between 1931 and 1948 in which the spleen had been examined histologically. This material comprised a wide range of the diseases encountered in a large teaching hospital.

RESULTS

Macroscopic findings

The weight of the spleen, recorded in 11 cases, ranged between 120 and 420 g. In 5 cases the weight represented a definite enlargement as compared with the average normal weight (Krumbhaar and Lippincott, 1939). No close relation, however, was found between the weight of the spleen and the incidence or severity of the lesions. In 3 instances the spleen was apparently normal in appearance and weight, though lesions attributable to periarteritis nodosa were found histologically. Middleton and McCarter (1935) also noted the occurrence of severe microscopic lesions of periarteritis nodosa in apparently normal or even atrophic spleens.

Fibrinous exudate, adhesions or focal thickening of the capsule occurred in five cases ; in three others capsulitis was evident only after histological study. The cut surface was usually firm but occasionally soft, and generally showed no distinctive changes. In two cases, however (cases 12 and 13), the cut surface was studded with small greyish-white foci which proved histologically to be intensely acute inflammatory lesions of the Malpighian bodies and trabeculæ respectively. In two instances (cases 5 and 10) the striking and unusual appearance of multiple infarcts was presented. Thus the macroscopic changes were neither uniform nor sufficiently characteristic to be of diagnostic value. In this connection the significance of multiple infarction is discussed later.

Histological findings

There were three cases (cases 1-3) in which no pathological changes were present apart from non-specific lesions such as congestion, slight cellular hyperplasia of the red pulp and hyaline arteriolosclerosis.

Disordered architecture. This was encountered in varying degree in six cases, sometimes due to widespread infarction (cases 5 and 10), sometimes to widespread destruction of Malpighian bodies and extensive trabeculitis (cases 8, 9, 12 and 13).

Vascular lesions. Characteristic lesions of periarteritis nodosa involving intra-trabecular arteries were present in nine instances

SPLENIC LESIONS IN PERIARTERITIS NODOSA

FIG 1 —Case 4. A splenic artery in the healed phase of periarteritis nodosa, showing intimal, medial and perivascular fibrosis with rupture and loss of the internal elastic lamina Elastic stain and Van Gieson. × 130.
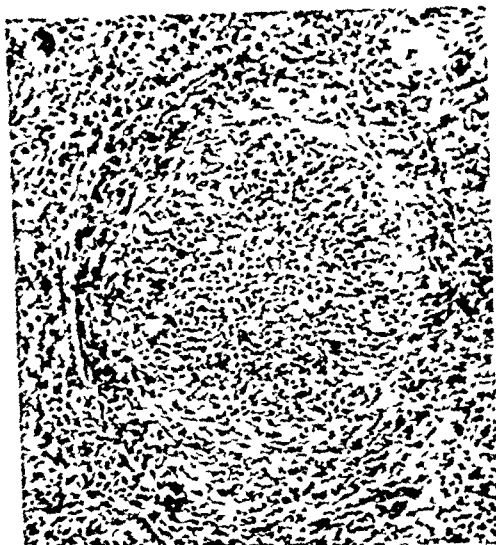
FIG 3 —Case 8 A Malpighian body lesion in the acute phase. Numerous polymorphs occupy the greater part of the follicle but a thin rim of lymphocytes remains. Hæmalum and eosin. × 130
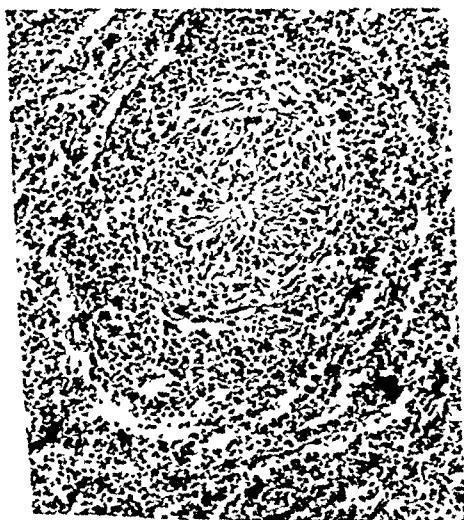
FIG 4 —Case 8 A healing Malpighian body lesion Fibroblasts are arranged radially at the centre and concentrically at the periphery A broad zone of lymphocytes surrounds the lesion. Hæmalum and eosin. × 145.

FIG 5.—Case 8. An almost completely healed Malpighian body lesion (A). The arteriole of the follicle is tangentially placed and shows no abnormality. Hæmalum and eosin. × 145.

(cases 4-12) and in five of these the arterioles of the Malpighian bodies were also affected. Lesions in the arterioles tended to be associated with the presence of fibrinoid material in the surrounding tissue (fig. 7). In the acute and subacute phase the lesions were of the classical type and do not merit special description (fig. 2). In the healed phase, however, inflammatory infiltration was absent or inconspicuous, the artery commonly showing intimal, medial and perivascular fibrosis, with rupture and segmental loss of the internal elastic lamina and the presence of iron-containing phagocytes in the fibrous tissue (fig. 1). Moreover, these characteristic changes were occasionally limited to one small segment of a vessel so that an accurate diagnosis could be made only after the study of many blocks cut in serial or semi-serial section. When, as in case 4, a lesion of this type occurs only very occasionally in an otherwise normal spleen, the necessity for careful histological examination in order to exclude periarteritis nodosa becomes apparent (Rothstein and Welt, 1933 ; Grant, 1939-42 ; Davson *et al.*, 1948). Similarly in case 6 a single infarct resulting from a single thrombosed aneurysm was the only abnormality detected.

In cases 13-16 vascular lesions of periarteritis nodosa were either absent, inconspicuous or of a type sufficiently unusual to present serious diagnostic difficulty, though other lesions were present which are referred to below. In case 13, for instance, an occasional intra-trabecular artery was seen to possess an apparently normal intima and media while the surrounding trabecular tissue showed fibrinoid necrosis with some polymorph and mononuclear infiltration and fibroblastic proliferation (fig. 9). Again, in case 14 the arterioles of the Malpighian bodies were involved in a tubercle-like lesion which is described below.

Trabecular veins were occasionally involved in trabeculitis. In two instances (cases 9 and 12) the endothelium of some of the intra-trabecular veins was raised by an infiltration with polymorphs and mononuclear cells, both vein and trabecular tissue being otherwise normal.

Capsulitis. Inflammatory changes in the capsule were present in eight cases. This lesion was characteristically focal. It occurred in the absence of splenic infarction and in the non-infarcted areas when infarcts were present. Typically, small areas of the capsule showed, in the acute phase, œdematous separation of the capsular fibres, especially in the inner aspect, and degrees of infiltration with varying proportions of polymorphs, lymphocytes and mononuclear cells. Later stages consisted of focal capsular fibrosis sometimes containing scattered lymphocytes and siderotic phagocytes. Case 12 showed both acute and chronic capsulitis, often in the same section. The finding of acute and healed phases of periarteritis nodosa in the same case or even in the same section is frequent in this disease. In two instances (cases 9 and 12) acute capsulitis was associated with linear

streaks of fibrinoid necrosis (in the deeper aspects of the capsule) in which definite arteriolitis could not be identified (fig. 6).

Trabeculitis. This was present in seven cases. Essentially it consisted of a small focal area of a trabecula which in the acute phase was swollen, œdematous and infiltrated by varying proportions of polymorphs, lymphocytes and mononuclear cells (fig. 8). The muscular and elastic tissues were destroyed and silver stains showed a marked increase in reticulin fibrils. The distribution of trabecular involvement varied considerably. In some instances (case 15) there were only occasional foci ; in others the trabeculæ were recognisable only with difficulty, the focal lesions having coalesced and replaced almost the entire trabecular system (case 9). In the later stages the lesions heal by replacement fibrosis. Similar trabecular lesions were described by Klinger. In five cases (8-12) the acute inflammatory infiltration was, in some areas, clearly a spread of a severe perivascular reaction to acute-phase periarteritis nodosa of the intra-trabecular arteries. In the remaining two cases (13 and 15) serial-section studies failed to demonstrate this association. Indeed, in case 13 trabeculitis was the predominant lesion and there was no other evidence in the spleen of periarteritis nodosa.

Malpighian-body lesions. These occurred in nine cases (cases 7-12 and 14-16). Three histological types were encountered. In the first type (cases 7 and 16) the centre of the Malpighian body was occupied by a small group of cells of epithelioid type, with or without associated arteriolitis. In the control series similar appearances were found in 17 cases comprising a heterogeneous assortment of disease entities ; hence this lesion is apparently unusual but non-specific. In the second type (cases 8-12) the Malpighian body, in the acute stage, was œdematous and contained at its centre a loose collection of polymorphs interspersed with fine strands of connective tissue. Fibroblasts were also present, usually radially arranged towards a thin peripheral ring of concentric connective tissue fibres. Surrounding the lesion there was commonly a rim of small lymphocytes marking the site of the original Malpighian body (fig. 3). In its most severe form (case 9) the cellular infiltration spread into the surrounding tissue, forming a lesion resembling a pyæmic abscess. Similar lesions were described in periarteritis nodosa by Klinger. In general, these foci of infiltration stood out clearly from the usually normal cellular content of the red pulp. The severity and distribution of these changes varied from case to case and acute and healed lesions were sometimes encountered in the same section.

In the third type (cases 9, 10, 14 and 15), masses of fibrinoid material of various sizes surrounded the arteriole which 'had itself undergone fibrinoid necrosis in some instances. Around the larger fibrinoid masses was a zone of fibroblasts and epithelioid cells, with occasional polymorphs and giant cells (case 14). The whole structure bore a superficial resemblance to a tubercle follicle but tubercle bacilli

SPLENIC LESIONS IN PERIARTERITIS NODOSA

FIG. 2.—Case 8. Acute-phase periarteritis nodosa of an intra-trabecular artery. Hæmalum and eosin.   × 90.
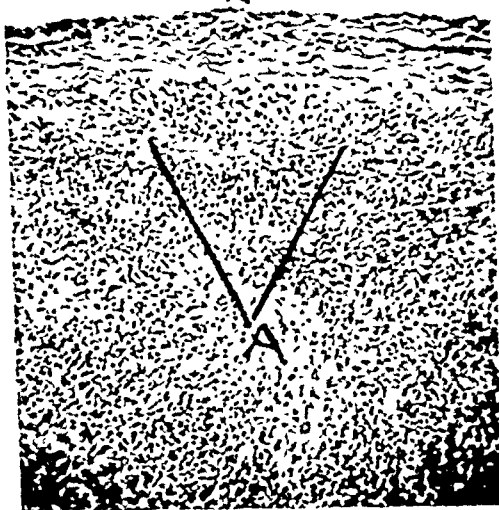
FIG. 6.—Case 9. Acute focal inflammatory infiltration of the splenic capsule with linear streaks of fibrinoid necrosis (A).   Hæmalum and eosin.   × 130.
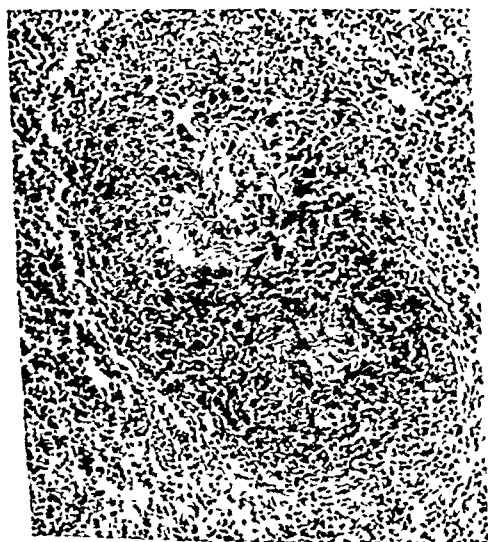
FIG. 7.—Case 9. Fibrinoid material in a Malpighian body associated with acute-phase periarteritis nodosa of the central arteriole. Hæmalum and eosin.   × 135.
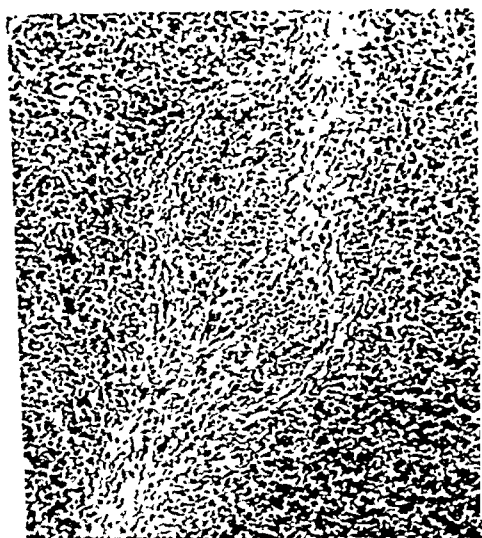
FIG. 8.—Case 11. Focal swelling and infiltration by polymorphs and mononuclears in a trabecula. There was no demonstrable associated vascular lesion. Hæmalum and eosin.   × 90.

were never found and the fibrinoid material stained strongly with Weigert's fibrin stain (fig. 10). This lesion was always infrequent. In two cases it occurred in the absence of concomitant readily diagnosable periarteritis. Granulomatous foci in periarteritis nodosa have been mentioned by Krahulik *et al.*, by Wegener and by Banowitch *et al.* (case 5). Occasionally this lesion may dominate the histological picture.

In the healing phase of the Malpighian-body lesions, most clearly depicted in the second type, the fibroblasts at the periphery were arranged concentrically, while those at the centre tended to be radially disposed, together forming a characteristic pattern. The completely healed lesion presented as a small fibrous nodule (figs. 4 and 5).

In some instances Malpighian-body lesions were clearly associated with acute periarteritis of the central arterioles. In many instances, however, this association could not be demonstrated in serial sections, the arteriole being not uncommonly eccentrically placed and showing no definite abnormality.

Pulp changes. Congestion and reticulum-cell and endothelial-cell hyperplasia of a degree commonly encountered in autopsy material were present in all cases. Moderately large numbers of eosinophils were seen in the red pulp in one case only, a finding also reported by Lamb (1914), Ophüls (1923) and Sandler (1938), but not mentioned in most of the published cases.

On the basis of these findings the sixteen cases may be divided into four groups.

Group 1. Three cases (cases 1-3) in which only non-specific lesions were present.

Group 2. Three cases (cases 4-6) in which the typical vascular lesions of periarteritis nodosa predominated.

Group 3. Six cases (cases 7-12) in which capsulitis, trabeculitis and Malpighian-body lesions co-existed with the typical vascular changes of periarteritis nodosa.

Group 4. Four cases (cases 13-16) in which capsulitis, trabeculitis and Malpighian-body lesions were present, while the typical vascular manifestations of periarteritis nodosa were either inconspicuous or absent.

DISCUSSION

Macroscopic changes

Although infarction is often stated to be frequent in periarteritis nodosa, it was by no means an invariable accompaniment of splenic involvement in our series. It has been recorded by Keegan (1925), Klinger (1931), Vance and Graham (1931) and Krahulik *et al.* (1935).

The two cases with multiple splenic infarction (cases 5 and 10) in our series are of special interest. They correspond to what has been described under the title of "Fleckmilz"—a word indicating multiple splenic infarcts or necroses occurring in the absence of an

obvious source of emboli. Schmeisser and Harris (1938) reviewed the 27 recorded cases of Fleckmilz and added two of their own. The condition appears to be of varying ætiology, occurring in eclampsia, chronic renal disease (Schmeisser and Harris), leukæmia, tularæmia (Simpson, 1928) and sulphonamide anaphylaxis (Black-Schaffer, 1945). In a case described by Magnus (1937) the lesion was primarily arteritic.

The two cases of multiple infarction in our series may thus be regarded as examples of Fleckmilz due to the microscopic form of periarteritis nodosa, a diagnosis which should be entertained whenever multiple splenic infarction occurs without an obvious source of emboli.

Histological findings

Capsulitis. The occurrence of focal capsulitis unassociated with infarction in eight cases suggests that it may be a feature of splenic involvement in periarteritis nodosa. It is not, however, a specific lesion. Klemperer et al. (1941) noted perisplenitis in half of their 20 cases of disseminated lupus and it has been reported in rheumatoid arthritis (Raven et al., 1948). Fox (1930), studying the spleen in a series of 25 cases of subacute bacterial endocarditis, found capsulitis to be frequent, whether infarcts were present or not. In our control series of spleens there were 15 cases of subacute bacterial endocarditis, and in 3 of these there was capsulitis unassociated with infarction. Splenic capsulitis does not appear to have been described in scleroderma.

Trabeculitis. Widespread focal trabeculitis was encountered only once in the control series of 400 spleens—in a case of subacute bacterial endocarditis. Occasional slight trabecular infiltration was seen in two other cases of the same disease. Klemperer et al., in their study of disseminated lupus, noted one case in which there was fibrinoid necrosis of collagen in a trabecula, but no lesions were found that closely resembled those occurring in our series. More et al. (1946) described trabeculitis in 6 of their 22 cases of sulphonamide allergy. However, we were unable to detect any evidence of sulphonamide hypersensitivity in the clinical histories of the 7 cases in our series which showed trabeculitis, and, apart from case 11 in which Guttæ " albucid " were used, there was no record that sulphonamide had been exhibited.

According to a recent statement of Zeek et al. (1948) splenic trabeculitis and arteriolitis are characteristic of cases of sulphonamide allergy and may be used as criteria for differentiating such cases from true periarteritis nodosa. Our results give no support to this view, since trabeculitis was prominent in all, and arteriolitis present in 4 of 7 cases in which sulphonamide hypersensitivity was not a complicating feature.

Trabeculitis does not appear to occur in scleroderma. Thus, although focal trabeculitis cannot be regarded as peculiar to periarteritis nodosa, its relative frequency in this series as compared with the control group suggests that it constitutes a definite manifestation of the disease.

SPLENIC LESIONS IN PERIARTERITIS NODOSA
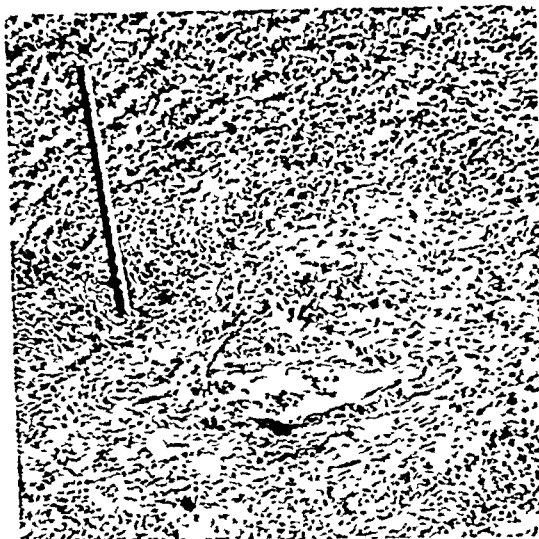
FIG. 9.—Case 13.   Fibrinoid necrosis and fibroblastic proliferation in trabecular tissue (A).   The intima and media of the artery appear unaffected.   Hæmalum and eosin.   × 100.
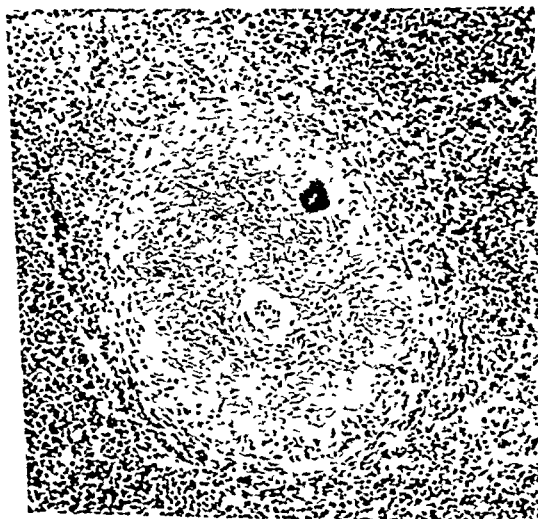
FIG. 10.—Case 14.   The central arteriole of the Malpighian body has undergone fibrinoid degeneration and the follicle is replaced by masses of fibrinoid material containing scanty degenerate nuclei.   A giant cell is present towards the periphery, where epithelioid-like cells occur.   Hæmalum and eosin.   × 110.

Although the lesion was commonly traceable to trabecular arteritis, in two instances (cases 13 and 15) it was present in the absence of demonstrable vascular lesions of periarteritis nodosa in the trabeculæ, and in these cases the origin of the trabeculitis was not apparent. It was thought to be analogous to the glomerular lesions of periarteritis nodosa, whose frequency has been emphasised recently by Davson et al., and to the skin lesions described by Macaigne and Nicaud (1932). In both the glomerular and dermal involvements, inflammatory and fibrinoid necrotic lesions occurred which were not attributable to the common vascular manifestations of periarteritis nodosa, and in both instances the authors ascribed the lesions to involvement of the capillaries. Spiegel (1936) has called attention to the fact that the skin (being predominantly a capillary bed) is an excellent " indicator " of the occurrence of capillary lesions in the disease. It is interesting to note that all seven cases showing trabeculitis were associated with diffuse necrotising glomerulitis, which occurred in nine out of the sixteen cases.

Malpighian-body lesions. Since changes comparable with the second and third types of Malpighian-body lesions were not seen in the control group, they may be regarded as supporting the diagnosis of periarteritis nodosa, even when, as occasionally happens, the typical vascular manifestations of the disease are not present in the spleen. Furthermore, when these lesions occur independently of arterial, arteriolar or venous lesions of periarteritis nodosa, the histological appearances support the view that, as in the case of trabeculitis, they may be regarded as manifestations of capillary involvement by this disease.

SUMMARY

1. Sixteen cases of periarteritis nodosa were divided into four groups on the basis of the histological findings in the spleen.

Group 1. Three cases in which there were no pathological changes apart from non-specific lesions such as congestion, minor degrees of cellular hyperplasia of the red pulp and arteriolosclerosis.

Group 2. Three cases in which typical vascular lesions of periarteritis nodosa predominated.

Group 3. Six cases in which changes such as capsulitis, trabeculitis and Malpighian-body lesions coexisted with the typical vascular changes of periarteritis nodosa.

Group 4. Four cases in which changes such as capsulitis, trabeculitis and Malpighian-body lesions were present but in which the typical vascular manifestations of periarteritis nodosa were inconspicuous or absent.

2. Trabeculitis and Malpighian-body lesions were often seen to be part of the inflammatory response to acute and subacute periarteritis nodosa of adjacent vessels. Serial-section studies showed that these lesions may, however, arise without this association, and

in such instances it is suggested that they had their origin in capillary periarteritis nodosa.

3. Focal capsulitis, trabeculitis and inflammatory lesions of the Malpighian bodies may be regarded as definite manifestations of the disease in the spleen, and suggestive of that diagnosis even when they occur in the absence of the typical vascular lesions of periarteritis nodosa.

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APPENDIX

SUMMARIES OF CASES

.Case 1 * (12). A man aged 25 who had been well until about 4 months before death, when he suffered from headaches and abdominal pain. Later he developed blurred vision. Blood pressure 200/150. While under observation he had a leucocytosis and an inconstant pyrexia. Before death his blood urea rose to 222 mg. per 100 c.c. and a tentative clinical diagnosis of periarteritis nodosa was made. At autopsy small thrombosed aneurysmal nodules were present on branches of the coronary, renal, hepatic, pancreatic and mesenteric arteries. The spleen (140 g.) appeared normal.

Histology. Typical acute-, subacute- and healed-phase lesions of peri-arteritis nodosa, with and without aneurysm formation, were present in the arteries of the myocardium, pancreas, liver, lung, kidney and mesentery. The spleen showed no abnormalities ; the kidneys the typical lesions of malignant nephrosclerosis in addition to those of periarteritis nodosa.

Case 2 * (10). A woman aged 26 who had intermittent swelling of the ankles for a year. Blood pressure 245/155. She developed pyrexia, leucocytosis and a pleural effusion and died suddenly. At autopsy small thrombosed aneurysms were found in the liver, pancreas and kidneys. Spleen enlarged (no weight recorded) ; cut surface dark red and firm.

Histology. Recently organised aneurysms were present in the liver and pancreas. Spleen normal. The kidneys were normal except for intimal fibroblastic proliferation of some intra-lobular arteries.

Case 3 * (3). A man aged 55, who, after a week's fever with abdominal pain, had a segment of gangrenous bowel removed surgically. A fortnight later he died in uræmia but with a normal blood pressure (120/80) Autopsy findings were not available.

Histology. The gangrenous bowel showed lesions of acute-phase periarteritis nodosa. The spleen contained an infarct but no vascular lesions were found. The kidneys showed widespread necrotising glomerulitis.

Case 4 * (13). A man aged 34 who had had rheumatoid arthritis of the fingers and hands about a year before death. The arthritis disappeared but he later developed headache, dyspnœa and weakness. Blood pressure 200/148. A clinical diagnosis of malignant hypertension was made. Before death the blood urea was 94 mg. per 100 c.c. There was no pyrexia or leucocytosis at this time. At autopsy left ventricular hypertrophy was present and the liver contained an area of necrosis 4 cm. in diameter. The spleen (200 g.) was firm and dark red on section.

Histology. The liver showed an infarct with an associated healing-phase lesion of periarteritis nodosa involving a branch of the hepatic artery. The spleen showed an occasional intra-trabecular artery in the late healed phase of periarteritis nodosa, but no other abnormality. In the kidneys some interlobar and arcuate arteries showed healed-phase lesions.

Case 5 * (1). A man aged 45 with a few months' history of pains in the limbs, chest and abdomen, loss of weight, pyrexia and conjunctivitis. Blood pressure 140/90. Leucocytosis was severe and the illness terminated in uræmia. At autopsy pericarditis was present and the lungs showed scattered infarcts. The spleen (120 g.) was adherent to the diaphragm. The outer surface was mottled bluish-pink ; the cut surface showed irregular dark areas on a pink background, suggesting widespread infarction.

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* Cases so marked have been previously reported, with special reference to the renal lesions in periarteritis nodosa. The number in brackets refers to the serial case number in that publication (Davson *et al.*, 1948).

Histology. Typical acute-phase lesions of periarteritis nodosa were present in the arteries of the lungs, kidneys and voluntary muscles. The spleen showed capsulitis, multiple microscopic infarcts and acute-, subacute- and healing-phase lesions of periarteritis nodosa of the larger intra-trabecular arteries. The kidneys showed diffuse necrotising glomerulitis.

Case 6 * (11). A youth aged 17 who had epigastric pain, vomiting and pyrexia, and abdominal tenderness. At laparotomy a portion of omentum was removed for histological examination. It showed the typical lesions of the acute phase of periarteritis nodosa. Blood pressure 132/84 ; blood urea 36 mg. per 100 c.c. General condition deteriorated and he died a month after the operation. At autopsy branches of the coronary and mesenteric arteries showed small nodules and both kidneys contained multiple infarcts. Spleen enlarged (weight not recorded) and contained one infarct.

Histology. Typical acute and subacute vascular lesions, with and without aneurysm formation, were present in the arteries of the heart, intestine, pancreas, adrenals, liver and voluntary muscles. The spleen showed an area of infarction and a thrombosed aneurysm in an intra-trabecular artery : the non-infarcted portions showed no abnormality. The kidneys showed infarcts secondary to thrombosed aneurysms in the larger arteries.

Case 7. A man aged 28 who had had headaches and dimness of vision for several months before death. Blood pressure 260/140. Small nodules were found on the radial arteries. Bilateral hypertensive retinitis was present, and several choroidal nodules typical of periarteritis nodosa were observed. A leucocytosis of 24,700 was present. Periarteritis nodosa with associated hypertension of malignant type was diagnosed. At autopsy small white nodules were present on branches of the coronary and mesenteric arteries ; the liver and kidneys contained infarcts. The spleen (150 g.) showed an area of peri-splenitis. The cut surface showed no abnormality.

Histology. Acute-, subacute- and healed-phase vascular lesions of periarteritis nodosa were present in the intestine, mesentery, pancreas and kidneys. The spleen showed healed-phase vascular lesions of the intra-trabecular arteries, occasional acute-phase lesions in the central arterioles, and, more frequently, epithelioid-cell foci in the Malpighian bodies. In the kidneys there were also present the histological changes of malignant nephrosclerosis.

Case 8. A man aged 63 who began to suffer from intermittent claudication about ten months before death. More recently he had had headache and œdema of the ankles. Albumin was present in his urine and the blood urea was 104 mg. per 100 c.c. Blood pressure 190/90. There was a slight leucocytosis and the urine contained red cells and leucocytes. At autopsy numerous small nodules were seen in the branches of the mesenteric artery and the liver contained several small nodules filled with red thrombus. Spleen not enlarged ; its surface showed a few small white patches ; the cut surface was mottled white and red throughout.

Histology. Acute- and subacute-phase vascular lesions of periarteritis nodosa were present in the lungs, pancreas, intestine and liver. The spleen showed capsulitis, trabeculitis, Malpighian-body lesions and acute- and subacute-phase vascular lesions in the intra-trabecular arteries. In the kidneys the arcuate arteries showed acute and subacute vascular lesions and some glomeruli showed partial fibrosis, others peri-glomerular granuloma formation.

Case 9 * (9). A man aged 52, whose illness began with conjunctivitis followed by weakness, dyspnœa, cough and sputum, pyrexia, albuminuria and leucocytosis, terminating in uræmia about ten months after the onset of his conjunctivitis.

At autopsy the lungs showed bilateral bronchopneumonia, and two small ulcers were present in the ileum. The spleen weighed 420 g. ; the surface showed adhesions. The cut surface was mottled white and dark red throughout.

Histology. Typical acute-phase vascular lesions of periarteritis nodosa were present in the ileum and in the peripelvic tissue of the kidneys. The spleen showed capsulitis with associated fibrinoid necrosis, trabeculitis, acute-phase vascular lesions of the smaller arteries, severe Malpighian-body lesions, and fibrinoid masses with epithelioid-cell and fibroblast reaction in the Malpighian bodies. Some veins showed subendothelial leucocytic infiltration. The kidneys showed diffuse necrotising glomerulitis.

Case 10 * (8). A woman aged 32, whose illness began with pain in the joints of her fingers followed by pneumonia and iritis, with pyrexia, albuminuria and leucocytosis. Five months later she died in uræmia.

At autopsy numerous infarcts were present in both kidneys. The spleen weighed 220 g. and contained a large number of infarcts.

Histology. The heart showed diffuse interstitial myocarditis. The spleen contained multiple infarcts and showed widespread trabeculitis and a few acute Malpighian-body lesions. The intra-trabecular arteries showed acute-phase periarteritis nodosa, the kidneys necrotising glomerulitis and occasional acute-phase vascular lesions of the intra-lobular arteries.

Case 11. A man aged 31 who, a year before death, had pain and swelling in the knees which cleared up satisfactorily. A few weeks before death he had pain in the joints, episcleritis and pyrexia. Blood pressure 120/60; there was no leucocytosis; the blood urea rose to 298 mg. per 100 c.c. The urine contained albumin, red cells and leucocytes. At autopsy two whitish areas of consolidation were present in the right lung; the jejunum showed multiple shallow ulcers and small nodules were present on the mesenteric artery branches. The spleen weighed 300 g.; its surface showed fibrinous adhesions; the cut surface was soft and brick-red, with whitish streaks.

Histology. Acute-phase lesions of periarteritis nodosa were present in the lungs, heart and jejunum. The spleen showed capsulitis and trabeculitis, many Malpighian-body lesions and acute-phase vascular lesions of the intra-trabecular arteries. Considerable numbers of eosinophils were present in the red pulp. The kidneys showed necrotising glomerulitis.

Case 12 * (4). A man aged 59, who had complained of weakness, sweating and occasional blood-stained sputum for several weeks. X-ray examination showed a patch of consolidation in one lung; pyrexia, leucocytosis and albuminuria were present. Blood pressure 120/60. At autopsy the lung apices showed appearances suggestive of chronic apical tuberculosis, the lower lobes patchy consolidation and scattered foci resembling tubercles. The spleen (wt. 400 g.) showed numerous minute white foci on the cut surface.

Histology. The lung apices showed areas of necrosis and fibroblastic organisation, and an occasional vessel showed acute-phase periarteritis nodosa. The spleen showed capsulitis with associated fibrinoid necrosis, trabeculitis and acute-phase vascular lesions of the central arterioles and Malpighian-body lesions. Some veins showed subendothelial leucocytic infiltration. The kidneys showed diffuse necrotising glomerulitis.

Case 13. A man aged 41 who had had bronchiectasis for many years. For two months before death there was hæmoptysis. When admitted he was seriously ill. Blood pressure 100/60. Œdema of the ankles was present. He died shortly after admission. At autopsy both lobes of the right lung contained many small white foci; the lower lobe of the left lung was bronchiectatic. Pericarditis was present. The spleen (150 g.) showed the cut surface closely studded with small white foci.

Histology. The lungs showed widespread focal necrotising alveolitis. Acute-phase vascular lesions were present in the liver and the peripelvic tissue of the kidneys. The spleen showed widespread trabeculitis but no typical vascular lesions. The kidneys showed diffuse necrotising glomerulitis.

Case 14. A woman aged 23 who had developed clinical pulmonary

tuberculosis about six years before death. This had responded satisfactorily to pneumothorax treatment. Six months before death' the blood pressure was 260/165, and her subsequent clinical course was that of rapidly progressive malignant hypertension. Tubercle bacilli had never been found in her sputum. At autopsy left ventricular hypertrophy was present and there were encapsulated caseous masses in the upper part of both upper and lower lobes of the right lung ; the left upper lobe contained numerous small calcified foci. The spleen (240 g.) was brick red and firm on section.   -

Histology. The lungs showed no evidence of ˙active tuberculosis but encapsulated calcified and caseous foci were present. Some pulmonary arteries showed the healed phase of periarteritis nodosa, and one branch of the pancreatic artery showed the healing phase. The spleen showed capsulitis, slight trabeculitis and occasional fibrinoid necrosis of the central arterioles with massive fibrin extravasation and epithelioid-cell reaction. The branches of the renal artery showed acute-phase vascular lesions with abundant perivascular fibrinous exudate and surrounding epithelioid-cell and fibroblast reaction.

Case 15. A woman aged 55 years who, three weeks before death, had " influenza " with cough and coryza, subsequently developed vomiting and pain in the chest. Slight sacral œdema was present. Blood pressure 160/80. A chest X-ray showed a small effusion at the left base. A leucocytosis of 20,000 was present. The urine contained red cells and albumin. The blood urea was 464 mg. per 100 c.c. At autopsy fibrinous pericarditis was present and a left pleural effusion was found. The spleen weighed 175 g. ; the cut surface appeared firm and congested.

Histology. The spleen showed capsulitis, slight trabeculitis and occasional fibrinoid necrosis of central arterioles. The kidneys showed diffuse necrotising glomerulitis.

Case 16. A woman aged 44 who came to autopsy with a clinical diagnosis of peripheral neuritis. No further clinical details available. At autopsy the small intestine presented acute ulceration throughout its length and the liver contained many yellow areas within its substance ; the kidneys contained numerous small infarcts.

Histology. Acute-phase lesions of periarteritis nodosa were present in a peripheral nerve and a healing-phase lesion in a hepatic artery branch. The spleen showed capsulitis and fibrinoid necrosis of central arterioles with surrounding zones of epithelioid cells. The kidneys contained numerous infarcts.

576 . 8 . 097 . 35 : 547 . 466 . 2 (glycine)

A COMPARISON OF THE DEGREE OF LYSIS BY GLYCINE OF NORMAL AND GLYCINE-RESISTANT ORGANISMS

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(PLATE CXLIV)

IT is an old observation in bacteriology that organisms can be trained to grow in concentrations of substances ordinarily lethal to them. Latterly the whole pioblem of drug resistance has come to have an increased importance because of its bearing on the use of antibiotics.

Gordon and Gordon (1947), showed that *Shigella shigæ* could be rendered resistant to concentrations of glycine up to 7·5 per cent. and that this resistance remained at a high level in subsequent sub-cultures. They further showed that resistance was not associated with the breakdown of glycine or the reduction of its concentration to the limits ordinarily permitting growth.

The mechanism of resistance acquired by organisms and its mode of action have been the subject of a good deal of experimental and theoietical study. Ehrlich (1907), who early recognised the resistance of trypanosomes to a series of organic arsenicals and dyes, suggested that in the resistant trypanosomes the specific drug did not enter the cell. Later, von Jancsó (1931, 1931-32) demonstrated by light sensitivity methods that organisms resistant to trypaflavine did not take up the drug, whereas susceptible ones did. In the case of *Trichomonas vaginalis*, however, Adler and Bichowsky (1948, personal communication) have shown that resistance to stilbamidine did not depend on non-entry of the drug, since its presence could be demonstrated in the resistant flagellate by fluorescent methods.

Dubos (1945) states that drug fastness may be " the result of progressive selection of the more resistant individuals occurring normally in any given population." It is likely that several factors may be involved in this resistance, either singly or together, of which the following three may be postulated :—(a) A difference in permeability of the cells, so that the drug may not be able to enter. (b) The production of a metabolite in excess, as for example in the formation of p-amino-benzoic acid in such amounts as to overcome the bactericidal effects of sulphonamides (Landy *et al.*. 1943 ; Landy and Gerstung.

1944). (c) A difference in enzyme constitution which confers the power of destroying the toxic drug, *e.g.* penicillinase-producing staphylococci.

Maculla and Cowles (1948) described how high concentrations of glycine caused lysis of organisms in certain conditions and it appeared to us that a similar technique applied to organisms rendered resistant to glycine might throw light on the mechanism of this resistance.

Experimental observations

The previous work on organisms rendered resistant to glycine was extended, using instead of *Shigella shigœ* various strains of *Bact. coli*. These organisms were made resistant by the technique previously employed of repeated subculture on media containing increasing concentrations of glycine over a period of several months. Eight strains of *Bact. coli* were used, namely four original strains and the corresponding four rendered resistant to glycine. Strain X in its resistant phase had become a non-gas-producing variant during 6 months' growth in broth containing glycine at concentrations steadily increasing up to 6 per cent. (Gordon, 1948). Strains 1, 2 and 4, grown in increasing concentrations of glycine on agar, were taken up to 7·5 per cent. during a period of five months. These still produced gas in the fermentation of sugars.

From broth cultures large volumes (usually 1 litre) were inoculated. The growth of the resistant strain X was poor at 24 hours and had to be allowed to grow for 48 hours. According to Maculla and Cowles, maximum lysability is found in very young cultures, but as comparison between cultures of young normal and old resistant cells had to be avoided, the control cultures were also grown for 48 hours. Strains 1, 2 and 4, both normal and adapted, grew adequately in 24 hours.

The cultures were centrifuged, the cells washed twice with distilled water, suspended in a small volume of distilled water (usually of the order of 20 ml. per litre of medium), and filtered through a small plug of glass wool to remove lumps. From this suspension 2-ml. samples were pipetted into a series of tubes ; one of these was treated with 4 ml. of distilled water, others with various glycine solutions, the total volumes being made up to 6 ml. Another sample was kept for nitrogen determination. All but the last mentioned were incubated at 37° C. overnight.

Next morning, *i.e.* after 16-18 hours' incubation, these suspensions were submitted to prolonged centrifugation and the supernatant fluids removed by pipette into a series of tubes, the volume taken being recorded. The protein which they contained was precipitated by the addition of one-quarter volume of 25 per cent. (w/v) trichloracetic acid. When the precipitates had flocculated—usually in 24 hours but sometimes longer—they were centrifuged out and thoroughly

washed twice with 5 per cent. trichloracetic acid. The washed protein was incinerated and the nitrogen determined by a semi-micro-Kjeldahl method, using Conway micro-diffusion units and the Conway micro-burette. The nitrogen content of the original suspension of bacteria was determined, and the precipitated protein expressed as a percentage of the total bacterial protein (table I).

TABLE I

The lysis of Bact. coli *by glycine*

Strain	Sample	Volume of supernatant fluid (ml.)	N found (mg.)	N per 100 ml. of bacteria (mg.)	Lysis (per cent.)	Lysis due to glycine (per cent.)
O	A	...	1·60	80·0	...	...
O	B	5·2	0·055	3·2	4	...
O	C	4·9	0·47	28·8	36	32
R	A	...	1·00	50·0	...	...
R	B	4·8	0·029	1·8	4	...
R	C	5·3	0·045	2·5	5	1

O = original strain 1.   R = resistant strain 1.

A = bacterial suspension (2 ml.).   B = trichloracetic precipitate after incubation of the suspension with water.   C = trichloracetic precipitate after incubation of the suspension with 6 per cent. glycine.

Different strains of bacteria were tested in this way (table II), and glycine concentrations in the neighbourhood of the lethal concentration for a normal strain were also tested (table III).

Discussion

Previous work on this subject (Gordon and Gordon, 1943, 1947) had shown that organisms grown on media containing moderately high concentrations of glycine present marked changes in the consistency of the colonies, *i.e.* they become mucinous and are difficult to emulsify. In the present work, bacteria growing in concentrations of glycine lethal to normal organisms showed similar but more marked characteristics on solid media. In fluid media they were not evenly dispersed, the cultures being of a granular nature. The changes which occurred might conceivably be associated with changes in the nature of the cell surface or membrane. There were also differences in the microscopic appearance of the normal and resistant strains (figs. 1 and 2), the resistant strain showing marked pleomorphism. Degenerate forms had been previously observed also in *V. choleræ* grown on glycine agar (Gordon and Gordon, 1943).

These considerations suggested that the difference between resistant and non-resistant forms might be one of permeability. The technique of Maculla and Cowles offered a means of demonstrating quantitatively any possible difference between the resistant and non-resistant strains.

The results reported above show that the resistant strains do in fact show an increased power of resisting lysis by glycine (tables I and II).

TABLE II

The reduced sensitivity of the resistant strains to lysis by glycine

Strain		Degree of lysis (per cent.)				Lysis due to glycine (per cent.)
		Glycine concentration (per cent.)				
		0	3	6	7·5	
X	O	1	...	...	12	11
	R	7	...	...	6	0
X	O	3	...	...	77	74
	R	8	...	...	25	17
X	O	1	...	...	17	16
	R	5	...	...	8	3
X	O	0	...	...	70	70
	R	0	...	...	25	25
X	O	1	63	...	...	62
	R	0	0	...	...	0
1	O	4	...	36	...	32
	R	4	...	5	...	1
1	O	0	...	19	...	19
	R	0	...	6	...	6
2	O	0	...	14	...	14
	R	1	...	0	...	0
2	O	4	...	12	...	8
	R	1	...	0	...	0
4	O	0	...	33	...	33
	R	0	...	11	...	11
4	O	1	...	15	...	14
	R	1	...	5	...	4

O = original strain.   R = resistant strain.

TABLE III

Variation in the degree of lysis of normal Bact. coli *(strain 1)
with various concentrations of glycine*

Glycine concentration (per cent.)	Lysis (per cent.)
0	0
1·5	8
2·0	16
2·5	16
3·0	15
6·0	19

PLATE CXLIV

BACTERIAL RESISTANCE TO LYSIS BY GLYCINE
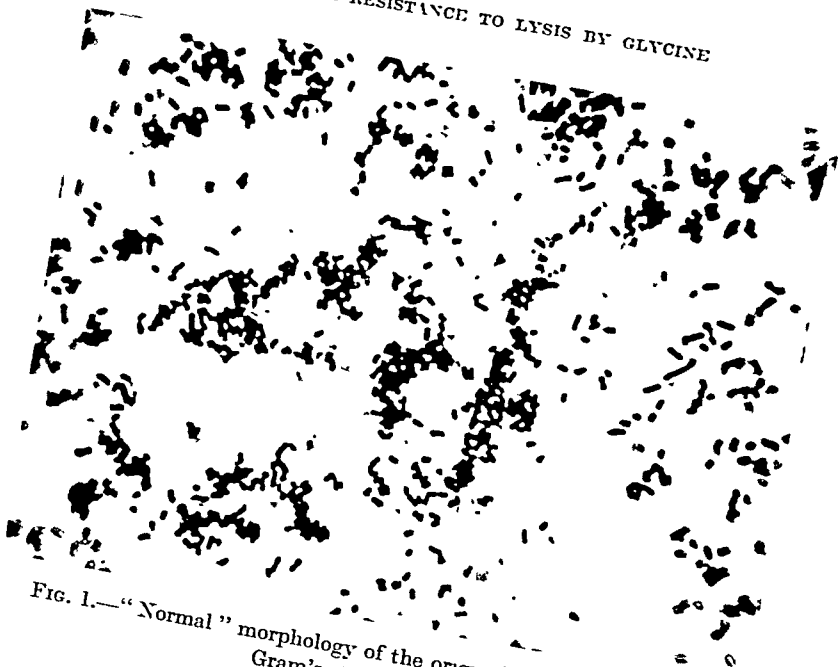
FIG. 1.—" Normal " morphology of the original strain of Bact. coli.
Gram's stain.   × 950.
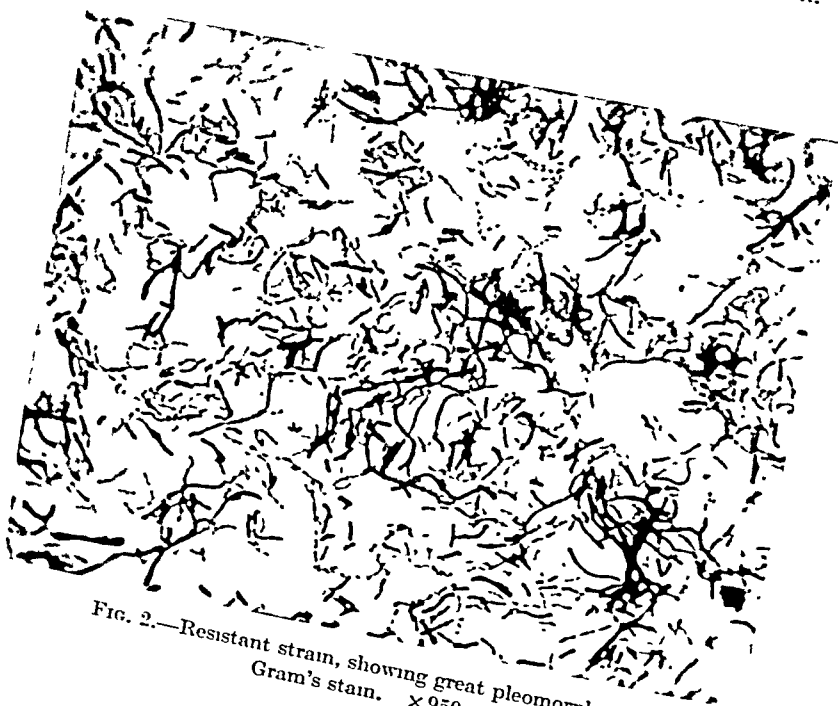
FIG. 2.—Resistant strain, showing great pleomorphism.
Gram's stain.   × 950.

That the inability of the original strain to grow in glycine is related to the lysis described by Maculla and Cowles is made probable by the results shown in table III, where the concentration at which the lysis occurs coincides with the lethal concentration (2 per cent.). The power of the resistant strains to grow at this and higher concentrations can apparently be attributed to their property of resisting the lysis, and this possibly involves diminished permeability to glycine. However, we cannot say that the increased resistance to lysis is necessarily the whole explanation of the phenomenon.

Summary

(1) Organisms adapted to growth in high concentrations of glycine show an increased power of resisting lysis by glycine.
(2) In normal (unadapted) organisms lysis begins at a concentration which coincides with that required to inhibit growth.

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PIGMENT PATTERNS IN EPITHELIAL TUMOURS OF THE SKIN

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(PLATES CXLV-CXLIX)

THE mere presence or absence of visible melanin is of little value in the classification of epidermal tumours. The melanomata were originally separated from the epithelial tumours on the basis of their pigmentation, and the separation has rightly survived the demonstration of the existence of amelanotic melanomata and of pigmented epithelial tumours. Within the group of pigmented epithelial tumours are several varieties, each of which can be exactly paralleled in behaviour and histology by a corresponding non-pigmented (or at least not obviously pigmented) form. So certain is this, that Becker, who has contributed as much (1927, 1930, 1934) to our knowledge in this field as anyone, concludes (1948) that " melanin in cutaneous carcinoma seems to have no real significance ". Stewart and Bonser's recent description (1948) of 57 pigmented epithelial tumours illustrates very clearly the distinction of these growths from melanomata and equally clearly the resemblance of their cases, in all respects except the presence of visible pigment, to the corresponding types of non-pigmented tumours.

The studies here recorded show that the presence of the readily visible melanin which leads to the labelling of an epithelial tumour as " pigmented " is merely an exaggeration of an extremely common process. Melanin in bulk may be the exception, but its complete absence is equally rare in many kinds of tumour. Even the most nordic of skins usually contains some melanin, and the occurrence of the same pigment in tumours derived therefrom cannot be regarded as surprising. But the melanin in fair skins is neither obvious to the naked eye nor readily seen in routine histological preparations, and to see the melanin in a lightly pigmented tumour one must either search laboriously in unstained sections or use special methods. It is solely because of this that the recognition of pigmentation in epithelial tumours has so far been largely confined to the more extreme grades. and a condition which is the rule for many kinds of skin tumour has been regarded as a rarity.

Given that pigmentation of epithelial tumours is common, the study of its distribution in an unselected series becomes at once of interest. It will be shown that the pattern assumed by the pigment in different sorts of tumours is much more characteristic than its amount. These patterns are of value in classification, and occasionally of practical use in biopsy diagnosis : their description is the main business of this communication.

It has, however, been impossible to avoid some examination of the mode of origin of melanin in these tumours. I believe that melanin is formed only within the melanoblasts and that the melanoblasts are of extra-epidermal and probably neural crest origin. Arguments on this matter may be left to the discussion, but the terms used in the descriptions which follow have been based on this belief. In all this work the assumption that the melanoblast may be regarded as an invading parasite or at least a symbiont has been a fruitful one, even if, for the cells of a single organism, the analogy cannot be pushed too far. The study of the reactions of a parasite to tumour formation in its host cannot fail to give valuable information about both parasite and tumour.

MATERIAL AND METHODS

This study is based on 311 biopsies of epithelial tumours of the skin from the routine material of this department. They include some multiple tumours and many serial biopsies during radiotherapy, and represent therefore only 226 patients. Many specimens of normal skin and of inflammatory lesions, dermal tumours and the like were also examined but are not specially considered here.

The method relied on throughout has been the silver technique of Masson. Bizzozero (1908) first showed in 1906 that melanin reduced silver salts with the deposition of metallic silver, so that even the smallest and palest granules of melanin become readily visible. Masson (1925) improved the method by using ammoniacal silver nitrate. The method is simple, flexible and reliable : it is a histochemical reaction whose simplicity will surprise and perhaps disappoint those used to the delightful uncertainties of silver impregnation. In the skin at least the reaction seems to be entirely specific : only the argentaffine cells of the intestine and the doubtful melanins of the colon and adrenal give the same reaction. The basic method is as follows :—

1. Fix in any ordinary fixative, preferably avoiding chromates.
2. Embed and cut in paraffin.
3. Bring to distilled water.
4. Leave overnight in 5 per cent. Fontana's ammoniacal silver nitrate, in the dark in a covered jar.
5. Rinse in distilled water.
6. Fix in 5 per cent. sodium thiosulphate for 1-2 minutes.
7. Counterstain for $\frac{1}{2}$-1 minute in carbol-safranin (Lendrum, 1947).
8. Wash briefly, dehydrate and mount in balsam (not D.P.X.).

Silver solution. Fontana's ammoniacal silver is probably best for ordinary use : the use of a slight excess of silver nitrate, decantation after 24 hours and filtration into the staining jar help to avoid deposit. Almost any silver solution can, however, be used in exactly the same way. Gomori (1948) has introduced a buffered hexamine silver solution for staining argentaffine cells which I have found worth while for melanin : it produces less deposit and, being nearly

neutral, detaches fewer sections from the slide. The following simplified version of Gomori's solution is recommended :—

Take 100 c.c. of 3 per cent. hexamine (called methenamine in the U.S.).
Add 5 c.c. of 5 per cent. silver nitrate. The precipitate re-dissolves.
Add 5 c.c. of borate buffer of approximately pH 8. (To 3 per cent. boric acid add a little phenolphthalein, then normal sodium hydroxide till a pink colour is just perceptible.)
Make up to 200 c.c. with distilled water.

Counterstain. This may of course be varied, but where most stains work badly carbol-safranin was found to be very satisfactory. It has a useful metachromatic effect with keratin, which it stains orange in contrast to the general rose-red.

HISTOLOGICAL OBSERVATIONS

Pigmentation of normal skin

Normal skin (fig. 1) treated in the way described above shows pigment in four sites :—

(a) In *phagocytic cells* in the dermis (melanophages). Parentheti-cally, I would like to make a plea for the avoidance of the word " melanophore " for these cells. Etymologically correct though the term may be, its use results in confusion with the melanophores of amphibia, which, though lying in the dermis, are Dopa-positive and homologous with the mammalian melanoblast. The only correct use of melanophore in mammals is for the cells of the Mongolian spot and the " melanophoroma " or blue nævus.

(b) In the *basal cells*, which usually form much the most heavily pigmented layer.

(c) In the *prickle cells*, which may be very lightly pigmented and whose pigmentation diminishes towards the surface (the mechanism of this is not understood). In both prickle cells and basal cells a cap of denser pigment often forms over the nucleus as though to protect it from the light.

(d) In *melanoblasts*. Though the silver stain, unlike the Dopa reaction, is not specific for melanoblasts, it is obvious on comparison of the silver and Dopa reactions on normal skin that the dendritic cells stained by both methods are the same. The long branched processes of melanoblasts, marked out by the fine granules of melanin within them, are unmistakable (figs. 1 and 6). Melanophages have much shorter processes and coarse clumps of melanin and no difficulty will be found in distinguishing them. For the present purpose, which involved the assembly of many old cases of which only paraffin-embedded material was available, the Dopa reaction was impracticable, but I believe that no errors in the identification of melanoblasts have resulted.

In normal skin, melanoblasts may be harder to see by this method than they are in pathological material. They are inactive, and the surrounding epithelial cells are laden with pigment produced long before ; consequently they are obscured. In irritated skin or the edge

of a healing ulcer, active melanoblasts are surrounded by epithelial cells they have not yet had time to pigment, and they may then be much more conspicuous.

Billingham (1948) has recently given a detailed description of the morphology of the mammalian melanoblast, with which my findings are in substantial agreement.

Frequency of pigmentation of tumours

There were 7 patients in this series with multiple tumours (2 with squamous carcinomata, 2 with squamous papillomata and 3 with rodent ulcers) but in each case all the tumours examined were similar in nature, and each may therefore be counted as a single case. The 226 cases dealt with fall into the following main categories :—

(i) Squamous papilloma—51 cases, of which 34 (67 per cent.) contained melanin.

(ii) Benign calcifying epithelioma—3 cases, all non-pigmented.

(iii) Squamous carcinoma—51 cases, none of them pigmented.

(iv) Rodent ulcer and related tumours—124 cases, of which 46 (37 per cent.) contained melanin.

These figures agree approximately with those of Becker (1934) except that he found 3 of 43 squamous carcinomata to be pigmented.

Patterns of pigmentation in tumours

Pigmentation in the tumours in which it was found was nearly always associated with the presence of melanoblasts. For the exceptions one of two reasons could always be given. On the one hand, there were six small biopsies of lightly pigmented tumours in which melanoblasts were presumably scanty and could not be found in the limited material available. On the other, there were very heavily pigmented tumours in which melanoblasts were largely obscured by the masses of melanin in the surrounding epithelial cells. It requires unusually dense pigmentation to do this : in only one of the present series (fig. 13) were melanoblasts completely obscured.

The pattern of the pigmentation depends chiefly on the distribution of the melanoblasts but also on the type of epithelial cells among which they lie. Three types of pattern can be recognised.

A Melanoblasts may lie among and be largely concealed by a heavily pigmented basal layer (fig. 4), the melanin content of the more superficial cells diminishing towards the surface.

B Melanoblasts may lie in much the same position, confined to the near neighbourhood of the basement membrane, but without any basal layer marked out by its pigment content and with little or no pigment in the epithelial cells generally (figs. 2 and 6). Melanoblasts are often large in these tumours, with very long processes, and because there is nothing in the surrounding epithelial cells to obscure them

PIGMENT PATTERNS IN EPIDERMAL TUMOURS
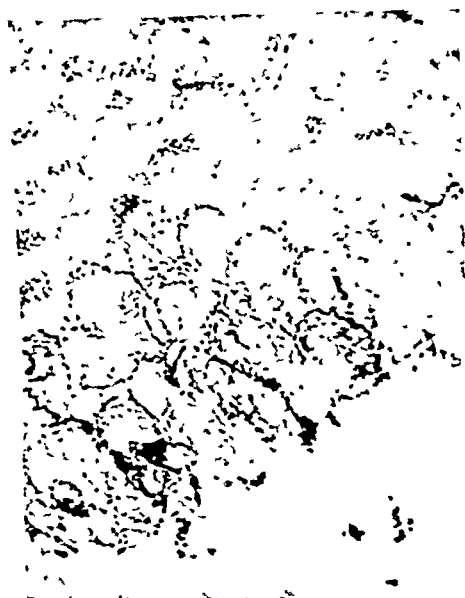
FIG 1.—Normal skin Melanoblasts and nuclear caps of melanin are well seen. Masson's silver method × 580

FIG. 2 —Squamous papilloma of groin in a man of 47 with unusually numerous basement membrane orientated melanoblasts (pattern B). Masson's silver method. × 220

FIG 3 — Squamous papilloma in a woman of 17 Hæmalum and eosin × 10
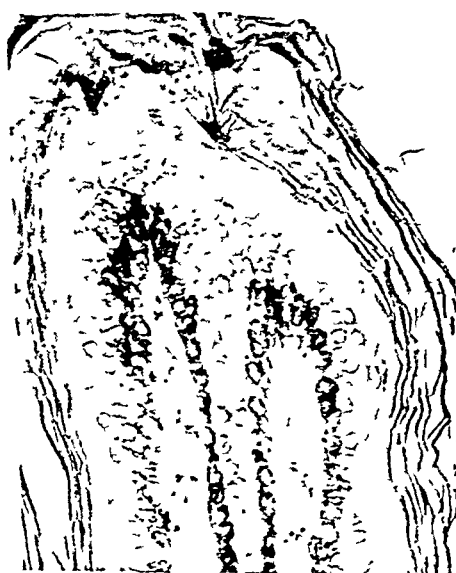
FIG. 4 —Same tumour as fig 3, showing the pigment pattern A of normal skin, with prominent basal layer and concealed melanoblasts Masson's silver method. × 235

they are more readily demonstrable by the silver method here than in any other ordinary pathological material.

C Melanoblasts may be scattered at random among groups of tumour cells : pigmentation of the epithelial cells is then more or less uniform, with little or no emphasis on the peripheral layer of cells (figs. 11, 12, 15, 16, 19). Pigmentation is often heavy and practically all tumours in which the melanin content is a conspicuous feature fall into this group.

Analysing these three patterns a little further, it appears at once that A is precisely the pattern of normal skin. Pattern B looks very much as if it was derived from A by disappearance of the more heavily pigmented basal layer. Pattern C seems to be the result of proliferation of the pigmented basal layer and disappearance of the less pigmented rete Malpighii. The normal habitat of the melanoblast is among the cells of the basal layer, and it might well be supposed that they would multiply more readily among cells of that type than among prickle cells.

This idea is strongly supported by a comparison of the types of tumour which show these three patterns. They will be considered in turn in the following sections.

Pigment pattern A

The tumours exhibiting this pattern form a small group of no great importance. There are only five examples in this series. All are squamous papillomata with irregular folding and heaping up of epithelium in which the structure of normal epidermis is very closely followed. All the layers persist and none is specially prominent. Figs. 3 and 4 show such a tumour. At first sight there is little difference between the lower power views of this tumour (fig. 3) and the commoner type of squamous papilloma (fig. 5), but a closer inspection shows the much greater thickness of the epithelium in fig. 3 and a comparison of the higher power views (figs. 4 and 6) shows that the increase is almost entirely on the part of the rete Malpighii. This pattern seems to be incompatible with rapid growth or with any degree of invasiveness.

Pigment pattern B

This is the characteristic pattern of the commoner form of squamous papilloma. Of the 51 squamous papillomata examined, 5 show pattern A and 5 pattern C : of the 41 which remain, the 24 which were pigmented all showed pattern B.

This group of 41 tumours appears to be a homogeneous one, characterised by a thickened folded epithelial layer whose dominant element is the rete Malpighii with its conspicuous prickle cells. Its members can be arranged in a series from impeccable benignity to incipient invasiveness, and an inverse correlation between pigmentation

and growth activity can at once be recognised. The most benign contain many melanoblasts, the most nearly malignant none. Fig. 2, with its numerous melanoblasts and some melanin in the epithelial cells, comes from a pedunculated tumour of slow growth. Figs. 5 and 6 show a rather more active, more sessile tumour which contains much sparser melanoblasts and only occasional granules of melanin in the epithelial cells. In the flat pre-malignant growths—the keratosis senilis of the dermatologists (Sutton and Sutton, 1939)—melanoblasts are infrequent or more often absent. The conclusion that the presence of the basement-membrane-orientated melanoblasts of this pattern are the mark of a benign tumour is greatly reinforced by the occasional occurrence of the phenomenon seen in fig. 7. This was taken from a case of keratosis senilis of the eyelid. Most of the tumour contained melanoblasts, but there were several foci of more active growth and in these no trace of pigment could be seen.

The borderline between the more active tumours of this group and the more benign squamous carcinomata is very difficult to draw. Failure to find pigment in any of the squamous carcinomata may therefore be taken as an extension of the process suggested above. Melanoblasts, it may be supposed, find that the more benign members of this group retain sufficient of the characters of normal epidermis to continue to offer a fairly congenial habitat, even if they are unable to secrete much pigment into the epithelial cells, but as the tumours acquire a higher rate of growth and differentiation falls the environment becomes increasingly unfavourable and melanoblasts become scantier and finally disappear. The point of disappearance in my material has always been well on the benign side of invasiveness, and that must be the usual event. It is conceivable that a larger series would show an occasional pigmented squamous carcinoma. A few of these have, in fact, been reported (Becker, 1934; Stewart and Bonser, 1948). One cannot, however, escape a suspicion that some of the reported cases may have been basal-cell carcinomata with partial squamous differentiation.

Pigment pattern C

This is the characteristic pattern of the rodent ulcer, but is encountered also in a small but important group of squamous papillomata. These 5 papillomata are all of the variety described by dermatologists as (among many aliases) verruca senilis (Sutton and Sutton). In spite of the occasional occurrence of doubtful forms I think the distinction of this variety of papilloma is a valuable one: in distribution, histology and prognosis it differs substantially from the squamous papilloma dealt with in the previous section and especially from the keratosis senilis with which it is most likely to be confused. For the present purpose its most important feature is the presence of large numbers of small dark-staining epithelial cells ("basal cells") and the absence or only limited development of the

PIGMENT PATTERNS IN EPIDERMAL TUMOURS

FIG. 5.—Squamous papilloma of face in a woman of 50. Hæmalum and eosin. ×13.
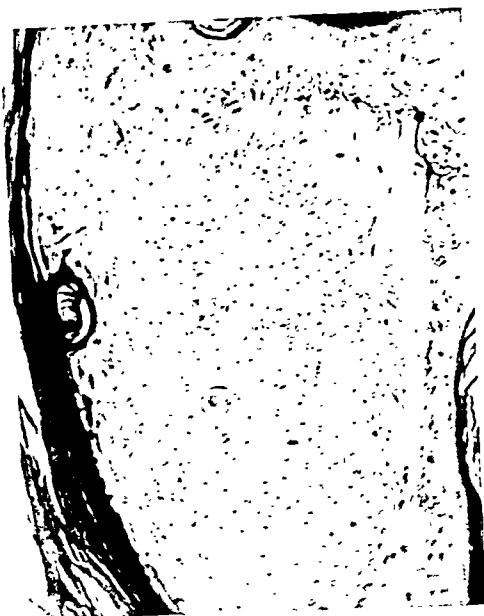
FIG. 7.—Keratosis senilis of eyebrow in a woman of 47 with foci of early malignancy. The bulk of the tumour was benign and contained melanoblasts (above and to right): occasional more active foci (to left) are without pigment. Masson's silver method. ×130.

FIG. 6.—Same tumour as fig. 5. Sparse basement-membrane-orientated melanoblasts and very little melanin in epithelial cells. A typical example of pattern B. Masson's silver method. ×320.

rete Malpighii. It is probable that most of the pigmented squamous papillomata described in the literature are of this type, *e.g.* Bloch's melano-epithelioma (1927) and Stewart and Bonser's case 17 (their figs. 1-3).

Tumours of this type are usually heavily and conspicuously pigmented. One of the present series (figs. 10 and 11) is an exception, in spite of its being well provided with melanoblasts. The pattern in these tumours tends to be a little less pure than in rodent ulcers, melanoblasts being sometimes more frequent along the basement membrane and the peripheral layer of cells a little more heavily pigmented, but such variation is usually associated with some degree of local squamous differentiation.

Of the 124 rodent ulcers examined 46 (37 per cent.) contain melanin, and in all of these the pattern is of this third type. Melanoblasts are scattered at random in the tumour islands. The epithelial cells usually contain granules of melanin (figs. 15 and 19) and there is little or no tendency to heavier pigmentation of the peripheral layer of epithelial cells. Only when the pigment is very scanty is it confined to melanoblasts: a picture such as that of fig. 16, with abundant melanoblasts among very lightly pigmented epithelial cells, is unusual. Really heavy pigmentation, of the kind that catches the eye at once in routine preparations, is uncommon in rodent ulcers. The tumour shown in figs. 17-19 is the only one in this series in which the pigment is unmistakable. It is one of a very large number which covered most of the abdomen of a woman of 62. Nearly all of those examined contained some melanin, but only this one tumour, which was not otherwise remarkable, was exceptionally heavily pigmented. As in many cases, the pigment which one noticed in hæmalum and eosin preparations was that in the phagocytes, not in the epithelium.

Only in the case of the squamous papilloma-squamous carcinoma series could a relation be established between pigmentation and activity of growth. This proved impossible for rodent ulcers. Pigment is absent from many slow-growing tumours and present in a few active ones. If one assumes an origin from various adnexa in the case of rodent ulcers, it is not surprising that only the tumours which arise from or imitate either the basal layer or the hair matrix should be pigmented; and it is probable that variations of this nature in the kind of differentiation are more important than its degree in determining the pigmentation of rodent ulcers. Studies on this and some other histochemical aspects of rodent ulcers are in progress and it is hoped to learn sufficient to justify a further communication.

Further observations

Some other points noted incidentally may be worth recording. One is the occurrence of a zone of de-pigmentation of the epidermis around some squamous carcinomata, especially the more malignant

(figs. 8 and 9). This seemed to be an active process of recent origin: melanophages containing melanin could still be recognised beneath the epidermis, which itself now contained none. Leukoplakia, of course, as its name implies, also shows de-pigmentation, and so did two cases of Paget's disease of the nipple. I have no cases of true Bowen's disease. Khanolkar (1947) reports two pigmented examples, but both were in Indians.

There were three cases in this material which corresponded more or less to the description of benign calcifying epithelioma, though none was calcifying. All were of the usual predominantly prickle-cell type and none contained melanin. Several cases of pigmented benign calcifying epithelioma have been demonstrated recently, notably by J. F. Heggie at a recent meeting of the Pathological Society, and there were two in Stewart and Bonser's series.

DISCUSSION

Practical uses of these observations

The silver method for the demonstration of melanin is so easy and reliable that it is well worth using for any additional information it may give in the biopsy of a doubtful skin tumour. Caution is necessary, however, in three respects. (a) These observations are based on a relatively small series of cases and are unlikely to have covered all the possibilities ; (b) they refer only to Europeans—Khanolkar's work suggests that conditions may be somewhat different in Indians (I know of no study in negroes)—and (c) the apparent absence of pigment is probably never a wholly reliable observation unless it can be shown that the adjacent skin is normally pigmented. The following conclusions, however, seem justifiable.

(a) The presence of a definite pigmented basal layer or of basement-membrane-orientated melanoblasts is strong evidence against malignancy in a squamous papilloma. In small tangentially cut biopsies in which invasiveness cannot be assessed, this may be of considerable value. Localised malignancy elsewhere in the tumour cannot of course be excluded by this means.

(b) An invasive tumour which contains melanin is almost certainly a rodent ulcer and not a squamous carcinoma.

(c) The generalisation that no pigmented epithelial tumour of the skin has ever been known to metastasise may be of value in prognosis.

(d) Though irrelevant to the main theme, it may be worth noting that one should never have any real difficulty in distinguishing between a malignant melanoma and a pigmented epithelial tumour. The appearance of fully differentiated melanoblasts, with delicate branching processes, lying among tumour cells of an entirely different type and without intermediate forms, is quite unlike that of the melanomata, which only exceptionally show really good dendritic

PIGMENT PATTERNS IN EPIDERMAL TUMOURS

FIG. 8.—Squamous carcinoma of face in a woman of 66. The two arrows mark the points of disappearance of melanin from the surrounding skin. Hæmalum and eosin. ×6.

FIG. 9.—Squamous carcinoma of scrotum in a man of 60. In this tumour, which is much more active than that of fig. 8, melanin disappears from the skin (arrow) much further from the tumour. Hæmalum and eosin. ×7.

FIG. 10.—Verruca senilis of face in a woman of 65. Hæmalum and eosin. ×8.
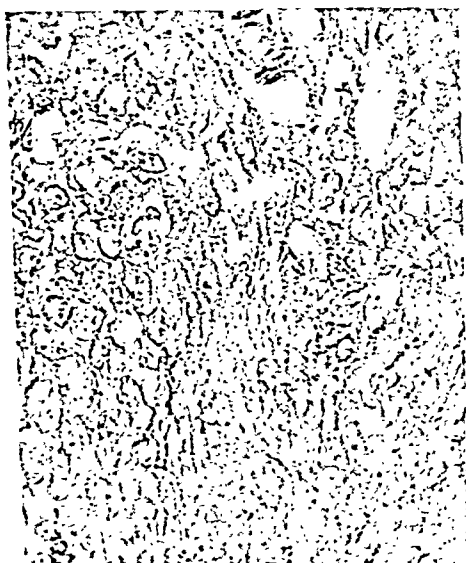
FIG. 11.—Same tumour as fig. 10. The tumour is unusually lightly pigmented, but melanoblasts are widely scattered through the tumour masses (pattern C). The most complete specimen is seen in the left lower quadrant. Masson's silver method. ×460.

PLATE CXLVIII

Fig 12 —Verruca senilis in a woman of 65. An average degree of pigmentation for this type of tumour, and with two melanoblasts visible but obscured. Masson's silver method. × 580.
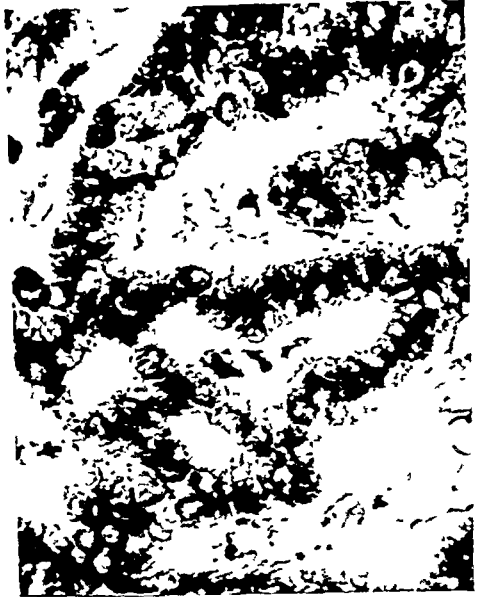
Fig 13 —Verruca senilis of forehead in a woman of 42 Unusually dense pigmentation, obscuring melanoblasts Masson's silver method. × 335.
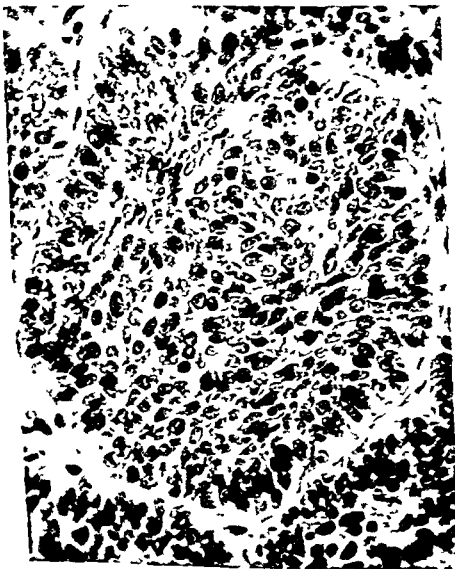
Fig. 14 —Rodent ulcer of temple in a man of 63. The kind of rodent ulcer which is nearly always pigmented Haemalum and eosin × 270
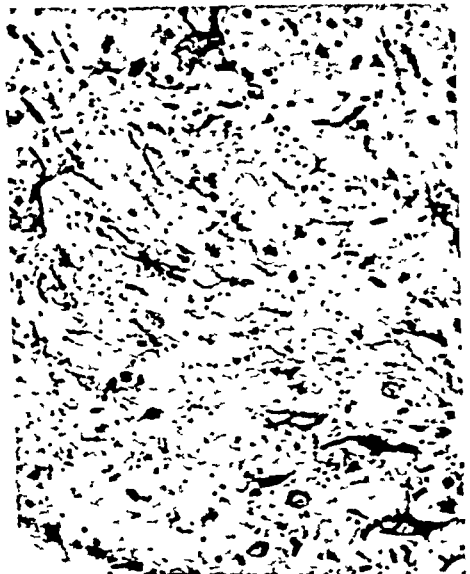
Fig. 15 —Same tumour as fig 14. Pigmentation of epithelial cells and scattered melanoblasts (pattern C) Masson's silver method. × 370.

forms. Further, the anaplastic squamous carcinomata, which alone are likely on general morphological grounds to be confused with melanomata, are never pigmented. Diagnosis of the amelanotic melanoma is another matter : occasionally, however, melanin can be demonstrated in an apparently pigment-free melanoma by the silver reaction.

The origin of melanin in pigmented epithelial tumours

It has been claimed that the epithelial cells of these tumours themselves produce melanin (Dawson, 1925 ; Stewart and Bonser, 1948). The demonstration of melanoblasts in such tumours (Becker, 1934 ; Khanolkar, 1947) does not of course at once refute this—some of the pigment may still be formed by the epithelial cells. Direct proof awaits the cultivation of epithelial cells and melanoblasts separately from such a tumour. The evidence provided by the present series on this point may be summarised as follows.

(a) It has been shown that melanoblasts can nearly always be found when melanin is present. The exceptions are explicable either because the melanoblasts were too scanty to be demonstrable in the material available or because they had produced so much pigment that they had become obscured.

(b) It has been shown that visible pigmentation of epithelial tumours is not an isolated phenomenon but the exaggeration of a common one, and that this common process has every appearance of being merely the extension of the ordinary process of pigmentation in normal skin to the tumour in question. What can be proved for normal skin may therefore be accepted as true for tumours also.

Such contradictory opinions have been expressed by the highest authorities on this and related matters (Dawson, 1925 ; Willis, 1948 ; and Stewart and Bonser, 1948 on the one hand : and Masson, 1926, 1948 ; and Becker, 1934 on the other may be taken as representative of the two main schools) that it is clear the examination of human pathological material cannot alone give a complete answer. But melanogenesis is a normal, not a pathological process, and its occurrence is not confined to man or even to vertebrates. The problem is one of the widest zoological significance and the pathological findings in the human subject must be interpreted in the light of zoological research. The last ten years have produced such abundant confirmation of earlier experimental work on the neural crest that few zoologists now seem to question the origin of skin pigment entirely from immigrant melanoblasts and of melanoblasts entirely from the neural crest. It is this, rather than any necessarily speculative (even if highly probable) deductions from the appearances seen in the present material that has led me to insist that the production of melanin in the tumours under discussion is a matter entirely of the melanoblasts.

Origin of melanoblasts

The zoological evidence for the origin of the melanoblast from the neural crest is sufficiently unfamiliar to be worth recapitulation. Much of it has been recently collected by DuShane (1948) and Rawles (1948). Some histological studies may be mentioned first. Becker *et al.* (1935) showed that only the melanoblasts of the skin are Dopa-positive : Bloch's earlier claim of a limited reaction by the basal cells was due to technical errors. Stearner (1946) has recently confirmed Ehrmann's work (1885) on the transference of pigment from melanoblasts to epithelial cells in amphibians. Billingham (1948) and Billingham and Medawar (1948) have made detailed studies of the guinea-pig melanoblasts and of their " infective " transformation which emphasise the secondary role of the epithelial element in pigmentation. These are all important, but the conclusive evidence is embryological.

(*a*) In *fish*, Borcea (1909) observed histologically the migration of pigmented cells from the neural crest. In higher vertebrates (and most fish) the migratory cells are not pigmented and cannot be directly identified in transit.

(*b*) In *amphibia*, transplanted or cultured whole neural tube (Harrison, 1910 ; quoted by Harrison, 1935) or neural crest (Holftreter, 1933 ; quoted by DuShane, 1948) forms pigmented cells. After excision of the neural crest early in development, pigment is lacking from corresponding segments in the adult (DuShane, 1935). When neural-crest grafts of different species are exchanged, the corresponding segment of the host acquires the pigment of the donor (DuShane, 1935).

(*c*) In *birds*, transplants or cultures of the neural crest form pigmented cells (Dorris, 1936). The course and timing of the migration of the neural crest cells have been followed in many grafting experiments (Eastlick, 1940 ; Willier and Rawles, 1940), and Watterson (1942) describes the actual invasion of the wing-bud epidermis by melanoblasts. One of Rawles's experiments (1945) affords particularly convincing evidence for the extra-epidermal origin of melanoblasts. The wing-bud of a 72-hour female White Leghorn embryo was transplanted to the coelom of a male Barred Rock embryo of the same age without ectodermal contact : there it grew and acquired host pigmentation, and at hatching was re-grafted to the back of another female White Leghorn chick : again it grew, and continued to produce feathers with the characteristic pigment pattern of a Barred Rock male.

(*d*) In *mammals*, the difficulty of operation on the foetus has prevented the elaborate and convincing experiments which have been possible on amphibians and birds. Rawles (1940) has, however, implanted foetal ectoderm of black mice into chick embryo coelom and succeeded in growing hairs. If the graft from a ten-day embryo

Fig. 16.—Rodent ulcer of external auditory meatus in a man of 77. Pigment almost confined to melanoblasts. Masson's silver method. ×205.
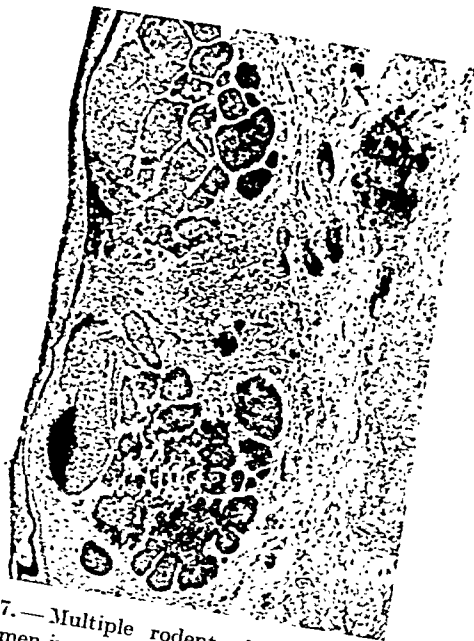
Fig. 17.—Multiple rodent ulcers of the abdomen in a woman of 62. Two of very many tumours shown. Hæmalum and eosin. ×33.

Fig. 18.—The lower of the two tumours of fig. 17. Dense pigmentation, obscuring the melanoblasts. The large black masses are phagocytes full of melanin, which were readily visible in routine preparations. Masson's silver method. ×370.

Fig. 19.—The same tumour, in a more lightly pigmented area : melanoblasts are readily visible. Masson's silver method. ×335.

included the neural crest, the hairs were pigmented, if it did not, they were colourless. She has more recently (1947) confirmed and extended these experimental findings. It seems improbable that a process established with such consistency in the rest of the vertebrate phylum, occurring at a stable phase of embryonic development when species differences are minimal, has been fundamentally altered during the evolution of the mammal. Even in mammals it will require some very strong factual evidence to shake the neural-crest hypothesis now.

<div align="center">SUMMARY</div>

1. The pigmentation of a series of epithelial skin tumours has been examined by a modification of Masson's silver method.

2. Of the squamous papillomata 67 per cent. and of the rodent ulcers 37 per cent. contain melanin : squamous carcinoma alone of common epidermal tumours is regularly without it.

3. Melanoblasts were demonstrable in pigmented tumours in nearly all cases : production of melanin by the epithelial cells of these tumours is considered improbable.

4. In pigmented tumours the pigmentation may assume any of three patterns :—*A*. A small number of squamous papillomata retain the structure and type of pigmentation of normal skin. *B*. Most squamous papillomata show melanoblasts along the basement membrane and little melanin in the epithelial cells. *C*. Rodent ulcers and squamous papillomata of the verruca senilis type have their melanoblasts scattered through the cell groups and their epithelial cells are uniformly and often heavily pigmented.

5. Embryological and experimental evidence for the neural-crest origin of the melanoblast is summarised.

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A CYSTIC HAMARTOMA OF THE LUNG IN A NEW-BORN INFANT

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(Plates CL and CLI)

Congenital cystic lesions of the lungs are generally regarded as uncommon. Published work on the subject consists almost entirely of reports of isolated examples, of which Koontz (1925) was able to find only 108. Holmes Sellors (1938-39), however, has reviewed 32 personally observed cases.

The manifestations of the condition are very diverse. There may be a single large cyst, or many cysts ranging in size from the microscopic up to 1 cm. or so in diameter, either forming a tumour-like mass or scattered diffusely throughout the parenchyma of a lobule, lobe or whole lung on one or both sides. The cysts may be confined to an accessory lobe, or may form a mass apparently arising from neighbouring structures such as the œsophagus or mediastinum. Seltzam (1905, quoted by Koontz) found a cystic mass of lung tissue under the left vault of the diaphragm. More than half the cases recorded have been in infants who either were born dead or lived but a short time after birth, but the condition is not necessarily incompatible with long life. In most cases which survive infancy, infection supervenes during childhood or adolescence.

Microscopically, the basic structure of these congenital cystic lesions is remarkably constant from case to case. The epithelial elements are of three types : (1) well-formed tubules resembling bronchioles, (2) more or less spherical cavities and (3) intermediate forms which are very irregular in shape, being tubular or cleft-like in one part and saccular in another. The epithelial lining of the cavities is a single or occasionally a double layer of cells showing all degrees of transition between low cubical and high columnar, and it rests on a well-formed basement membrane. Goblet cells are rarely present and there may be mucous glands in association with some of the larger tubules. The basement membrane is supported by a layer of plain muscle and often elastic tissue. Cartilage may be present in the stroma, either in association with bronchioles or

arranged in tumour-like masses. The cells of the supporting stroma are less differentiated than in normal lung tissue. The blood vessels are small and not numerous, and they bear no constant relationship to the epithelial structures. Anthracotic pigment is absent from congenital cystic lesions, a feature which has been of use in deciding whether a given cystic structure is congenital or acquired.

In view of the wide variety of appearances which may be presented by congenital cystic disease, especially when complicated by inflammatory changes, it is not surprising that cases have been described in the past under a variety of titles—fœtal cystic adenoma, bronchial adenoma, cystic degeneration, " honeycomb lung " and congenital bronchiectasis. Even these terms do not fully convey the bewildering variety of theories which have been suggested to explain the presence of the cysts.

Broadly speaking, the different views on the genesis of congenital cystic disease of the lung may be classified under three headings :—

(1) The condition is to be regarded as neoplastic or at least semi-neoplastic. Esch (1928), for instance, describing what he calls a congenital adenoma in a new-born infant, considered it to be a " diffuse hamartoma."

(2) The cyst formation is the result of syphilitic or other inflammatory bronchial stenosis which has caused bronchiectatic dilatation more distally. But many cases show no evidence of inflammatory reaction and where this occurs it is more likely to be secondary or coincidental. Pappenheimer's (1913) case is of interest in this connection. He found a cystic right upper lobe in a premature infant whose blood-Wassermann reaction was strongly positive, but the lungs showed no evidence of syphilis.

(3) The condition is a developmental defect. The great majority of writers have come to this conclusion, but they differ in their interpretation of the mechanism by which the developmental error is brought about. Koontz, for instance, found constrictions at the junction between tubules and cysts in his case. He inferred that the cysts were caused by the damming up of secretions distal to the constrictions. Wolman (1922-30) in his case found no such constrictions and thought that the obstruction was due to the absence of any communication between the cystic mass and the bronchial tree. Also the fact that few alveoli were present led him to believe that bronchial proliferation had occurred at the expense of alveolar formation. Pappenheimer, on the other hand, found by serial sectioning that the dilated spaces were in direct communication with bronchi on the one hand and alveoli on the other. Both he and Miller (1926) deny that the microscopic appearance of the epithelial structures is compatible with any great degree of distension of the cavities by pressure from within. Many writers have suggested that, in some cases at least, aplasia of the alveoli has led to mechanical dilatation of the bronchi by respiratory pressure. It is not always

clear from the descriptions, however, that mature alveoli were present as part of the lesion and not merely as part of the normal lung tissue. For full discussions of these and other questions the reader is referred to the articles of Koontz and of Holmes Sellors mentioned above.

CASE REPORT

Maternal history

The mother, an American aged 20, was 28 weeks in her first pregnancy when admitted to hospital on account of acute hydramnios of ten days' duration. Up to that time her pregnancy had been normal and uneventful and she seemed perfectly healthy. Paracentesis succeeded in removing only 600 c.c. of fluid mixed with blood, so the membranes were ruptured artificially, releasing a large quantity of fluid. Ten hours later the patient was delivered of a male child weighing 4 lb. 6 oz. (2 kg.). The infant was cyanosed and limp, and died in fifteen minutes, respiration never having been properly established. The placenta and membranes were expelled spontaneously and were normal except for a small fresh retroplacental blood-clot thought to be due to the attempted paracentesis. The cord was of average length but twice the average thickness.

Both the patient and her husband gave a negative Wassermann reaction. The mother was group O, Rh-negative; the father group A, Rh-negative. There were no known Rh antibodies in the mother's serum.

Post-mortem examination

The infant was of normal size for the 28th week, well proportioned and not jaundiced. Abdominal distension was easily apparent on inspection. The stump of the cord was about twice the expected thickness.

When the chest was opened the mediastinum was found to be deflected well to the left, apparently by a very large inferior lobe of the right lung. The size and position of the right upper and middle lobes were normal. The heart lay against the wall of the left chest in the region of the axilla, with the collapsed left lung above and behind it and the right lower lobe in front and below. The apparent enlargement of the right lower lobe was found to be due to the presence of an almost spherical pinkish-grey tumour-like mass (fig. 1), 6 cm. in diameter, presenting from the mediastinal aspect of the lobe, which was stretched out to form a shell of lung tissue on its anterior, lateral and superior aspects.

The mass had a firm rubbery consistency and was non-crepitant. Its surface was studded with rounded elevations up to 0·5 cm. in diameter. The cut surface was whitish, relatively bloodless and coarsely trabeculated. Inspection with a hand lens revealed a fine honeycomb texture between the trabeculæ, and there were a few large cystic spaces containing clear, sticky fluid. The free surface of the " tumour " had an investment of visceral pleura continuous with that on the lower lobe. After cutting through this membrane at the junction of the tumour and the lung tissue, it was possible by

gentle blunt dissection with the finger-tip to shell out the mass from its investment of lung tissue. Numerous thread-like blood-vessels passed across between tumour and lung. Careful search revealed only one channel of communication between the tumour and the bronchial tree. This was a narrow bronchial tube springing from the caudal aspect of the right main bronchus close to its origin from the trachea. It was 2 cm. long and its external diameter was considerably less than that of the bronchi passing to the normal lobes of the lung. It was quite patent at its origin but the lumen narrowed as it approached the tumour until at the distal end it would only admit a stout hair. India ink injected into the bronchus was seen on subsequent microscopic section to have entered some of the spaces of the tumour. The bronchus was accompanied by a branch of the pulmonary artery.

Of the other viscera, the heart and great vessels had a few punctate hæmorrhages on their surface but were otherwise normal. The thymus was normal in size and position. The left lung was airless, the upper and middle lobes of the right lung partly expanded. The right lower lobe, stretched and compressed though it was, also had air in its alveoli. The abdominal distension was due to the presence of deep yellow, clear fluid in the peritoneal cavity. The liver was very dark, had a tense capsule and bled freely when incised. There were no abnormal appearances in any of the other organs except for moderate congestion, but the tissues of the abdominal wall were obviously œdematous. The skull and its contents did not show any abnormal appearances. The brain had not yet formed the secondary convolutions.

Microscopic appearances

The " tumour " was composed of irregular, epithelium-lined spaces separated by a scanty cellular stroma (fig. 2). The spaces varied considerably in size and shape and in the nature of their epithelial lining. On the one hand there were straight tubules with a regular outline indistinguishable from fœtal bronchioles. On the other there were larger spaces, the outline of which was irregular, apparently because of distortion by pressure of neighbouring structures. Between these two extremes were spaces which had an irregular outline because of diverticula projecting from their walls, an appearance which strongly suggested an attempt at budding and branching. There was no sharp line of demarcation between these different kinds of structure ; indeed some of the single elongated spaces were obviously tubular at one end and cyst-like at the other, with irregular diverticula in between. The structures which were regular in outline and resembled fœtal bronchioles were themselves of two kinds. The larger, which were few in number, had a lining of narrow compact columnar cells with elliptical nuclei situated more or less centrally. The basement membrane was well formed and was supported by a layer of plain muscle and some elastic fibres. The other bronchiolar tubules had a

PLATE CL

CYSTIC HAMARTOMA OF LUNG

FIG 1.—Right lung viewed from the posterior aspect, showing the large cystic tumour-like mass projecting from the mediastinal surface of the lower lobe. The strip of cardboard lies behind the bronchus leading to the tumour and has a branch of the pulmonary artery emerging below it. ×1·8.

smaller calibre and were lined by a single layer of regular, broad cubical cells. The cytoplasm of these cells remained unstained and their nuclei were spherical and situated against the free border of the cell (fig. 3). A basement membrane was present in some of the tubules, but in others the epithelium lay in direct contact with the surrounding stroma. The deformed cyst-like structures and the irregular branching cleft-like spaces possessed a lining of epithelial cells which was far from being uniform. For the most part, especially in the larger spaces, the lining was of low cubical cells with clear cytoplasm ; elsewhere it was columnar, with central nuclei, and there were transitions between the two kinds of cells. There was yet a third type of epithelium quite distinct from the other two. Its cells were long and cylindrical, their cytoplasm staining only faintly with eosin, their nuclei elliptical in the long axis and situated at the basal end. The staining characteristics were also distinct in that the cytoplasm stained deeply with mucicarmine, with pyronin and with safranin. Some of the large spaces, particularly those near the centre of the tumour, were completely lined by epithelium of this type, and in these the epithelium invariably covered an area larger than its basement membrane, the sheet of cells being thrown into folds which projected into the lumen of the cavity and sometimes filled it. Many of the cells in these circumstances had desquamated into the cavity and had undergone degenerative changes. This was particularly noticeable where the vesicles were so closely packed together that there was little or no intervening stroma. Some adjacent vesicles of this type even had a common basement membrane ; others consisted simply of a sac formed by the basement membrane and filled with disintegrated cells. The long basophilic columnar cells were also present in some of the spaces lined mainly by cubical epithelium. Here they formed papillary tufts projecting into the lumen, with cubical cells on either side of them, the transition between the two being quite abrupt (fig. 4). Because of the ease with which the long columnar cells could be identified in sections stained with hæmalum and pyronin (an observation for which I am indebted to Mr S. Nicholson of this laboratory) it was possible to trace their development. In places in such sections an angular projection of cubical epithelium had at its apex three or four slightly larger cells which were basophilic. This appeared to be the earliest detectable stage in the development of the papillary tufts.

The stroma of the tumour consisted mainly of undifferentiated cells with oval or round nuclei. Plain muscle formed an investment round most of the tubular spaces. Staining with osmic acid and by Verhoeff's method showed that elastic tissue was present round most of the epithelial structures. There was no definite evidence of cartilage formation. Blood vessels were few and poorly differentiated, being mainly capillaries containing (in cross section) one or two corpuscles. In places capillaries could be seen encroaching on the basement

membrane of the smaller spaces. There were a few cleft-like lymphatic
channels. The trabeculæ which divided the tumour into lobules were
composed of small irregular cells separated by an unstained matrix.
When stained deeply with safranin these cells showed fine filaments
radiating outwards in all directions into the surrounding matrix,
and in many places the filaments formed tangled masses by inter-
twining with filaments from neighbouring cells. Here and there in
the trabeculæ were thin-walled vessels, some of which had ruptured,
allowing blood to escape into the matrix in such a way as to suggest
that this matrix had been semifluid during life. Sections cut from the
region between the tumour and the right lower lobe showed that the
tumour was covered by a thin pseudo-capsule composed of tissue
similar to that of the trabeculæ. This was traversed by many fine
blood-vessels but not by bronchioles.

Sections from the lungs showed in general a normal architecture
but with varying degrees of failure to expand. The other organs
showed nothing of note. There was no evidence of cystic disease
in any of them.

DISCUSSION

There can be little doubt that the epithelial structures of which
the mass is composed are of bronchiolar origin. The mass is a space-
occupying lesion distinct from the lung parenchyma; it does not
replace any part of the normal respiratory apparatus and it has its
own bronchus. It may therefore be regarded as an attempt at the
formation of an accessory lobe. The position of the mass between
the right lower lobe and the mediastinum is similar to that of the so-
called azygos lobe, a structure normally present in quadrupeds and
sometimes found in man in vestigial form (Keith, 1948).

The microscopic structure of the mass is significant when considered
in relation to the development of the normal lung. In early fœtal
life the lung is composed of branching tubules lined by a single or
double layer of columnar epithelium and lying in a cellular stroma.
After the 16th week smaller tubules branch out from the bronchioles
and end in saccular dilatations. These tubules and their terminations
are lined by cells which, as Barnard and Day (1937) have pointed out,
are quite distinctive in appearance and are not normally seen in the
mature expanded lung. The cells are cubical, arranged with diagram-
matic regularity, and have clear cytoplasm which remains for the most
part unstained in hæmatoxylin and eosin preparations. The nuclei
lie against the free border of the cell in the tubular off-shoots, but
tend to lie more centrally in the dilated terminations. These termina-
tions are destined to form the mature alveoli. The process begins
about the 20th week, when capillary loops encroach on the cubical
epithelium and will later penetrate between the cells, causing the
vesicles to become transformed into mature though unexpanded
alveoli.
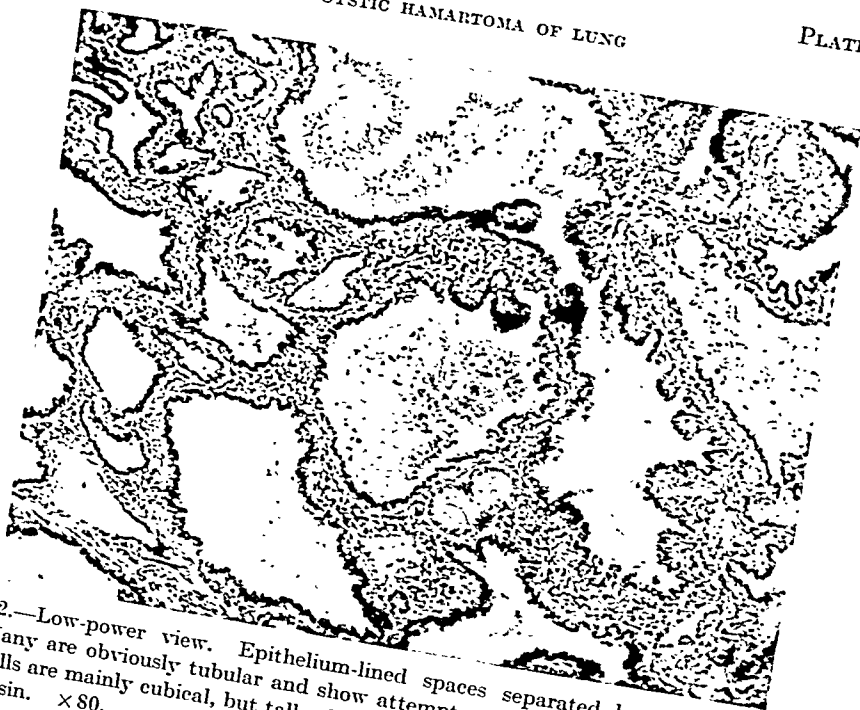
FIG. 2.—Low-power view. Epithelium-lined spaces separated by cellular stroma. Many are obviously tubular and show attempts at budding and branching. The cells are mainly cubical, but tall columnar cells are seen in places. Hæmalum and eosin. ×80.

FIG. 3.—High-power view showing well-formed tubules of the type seen in the nineteenth week of fœtal life. Hæmalum and eosin. ×270.

FIG. 4.—A papillary tuft of mucus-secreting cells is shown projecting from cubical epithelium. ×270.

In the " tumour " under discussion these types of epithelial structure are clearly represented. The tubular offshoots of the fœtal bronchioles which are characteristic of the 19th or 20th week of fœtal life are numerous and many are well formed. It is clear that the spaces of which the mass is composed are distorted and irregular overgrowths of these tubules and their dilated terminations. Moreover, although the stage of commencing capillary encroachment has been reached, in no place is there actual invasion of the epithelium by the capillary loops.

It is thus possible to assign to the tumour a " developmental age " corresponding approximately to the 19th week of fœtal life. At this stage the progress towards maturity of the epithelial elements must have ceased, for there are no structures present which correspond to a later period. Proliferation, however, had proceeded in an irregular manner, and at such a rate that the mass became larger than any of the normal pulmonary lobes. Presumably its growth would have ceased had the infant survived for a certain length of time.

A feature of the tumour which calls for further comment is the presence of the long, mucus-containing columnar cells which form a plicated lining to some of the spaces and occur as isolated tufts in others. Such appearances are not normally found in either the fœtal or the adult lung, but they do occur in certain pathological states. They are not uncommon, for instance, in chronic inflammatory conditions such as tuberculosis, bronchiectasis and the pneumoconioses. They have been described in the lungs of experimental animals which had been subjected to the action of irritants (Gazayerli, 1936), and they are a characteristic of the disease of sheep known as epizootic pulmonary adenomatosis, jaagsieckte or Montana progressive pneumonia (Cowdry and Marsh, 1927 ; Dungal. 1937-38). A similar pulmonary adenomatosis has been described in man (Richardson, 1940, case-report), while Simon (1947) gives a full bibliography of the disease in man and animals. In all these conditions it is thought that the mucus-secreting cells arise by metaplasia of adult alveolar epithelium. How they can occur in a congenital malformation when there has been neither irritation nor infection is a matter for conjecture, but the fact that the cells are most numerous at the centre of the tumour where the blood supply is poorest is rather suggestive.

SUMMARY

A cystic hamartoma of the lung in a premature infant is interpreted as a malformed accessory lobe in which arrest of maturation had occurred at a stage corresponding to the 19th week of fœtal life without diminution in the rate of growth, the resulting mass presenting appearances like those of a benign neoplasm. An unusual type of metaplasia of the epithelial cells of the hamartoma is described.

I am indebted to Dr Eric Wordley, Director of the department, for much helpful advice and criticism, to Mr Austin Concanon for the clinical details, to Miss J. P. Butler and Mr A. J. Short for the photography, and to Messrs Ilford Ltd. for the photomicrography.

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THE EFFECT OF ANTERIOR PITUITARY EXTRACT IN ALLOXAN DIABETES

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(Plates CLII-CLIV)

Dunn, Sheehan and McLetchie (1943) demonstrated how alloxan produces selective necrosis of the pancreatic islets in the rabbit, and a diabetic condition in the same species following the administration of this substance was reported by Bailey and Bailey (1943), Hard and Carr (1944) and Kennedy and Lukens (1944). The permanence of established alloxan diabetes was further suggested by the fact that Kennedy and Lukens observed the condition over long periods up to nine months. Ogilvie (1944, 1944-46), on the other hand, has shown that brief treatment of the rabbit with a crude anterior pituitary extract, while producing transitory diabetes, results in pancreatic islet enlargement to an extent which approximately doubles the amount of islet tissue. Alloxan and crude anterior pituitary extract are thus diametrically opposed in that they cause respectively destruction and growth of the islet tissue in the rabbit. Accordingly the idea was conceived of making rabbits severely diabetic with alloxan and then administering anterior pituitary extract with a view to alleviating the diabetic condition through the pancreotropic action of the extract.

MATERIALS AND METHODS

Alloxan (100 mg. per kg. body weight) was given intravenously as a 5 per cent. solution in sterile saline to induce a persistent diabetes. The anterior pituitary extract, prepared by the Glaxo and Organon Laboratories Ltd. after the method of Young (1938), was a crude saline product of fresh ox anterior pituitary glands, made up so that 4 c.c. were equivalent to 1 g. of gland. It was tested bacteriologically for sterility, and, although prepared at a point near freezing, was thereafter stored at room temperature. The extract was given by the subcutaneous route with precautions as to sterility.

The animals used were English rabbits. They were kept in metabolism cages and given daily 150 g. of a mixture of 40 per cent. oats, 30 per cent. bran and 30 per cent. maize, 300 g. of cabbage, 15 g. of hay, and water *ad lib.* The energy value of this diet was calculated by analysing its constitution as regards carbohydrate, protein and fat and applying the usual factors 4·1 × 9·3. Daily measurements included body weight, food consumption, urinary sugar, urinary

volume and, when necessary, blood sugar and urinary ketones. Blood sugar was estimated by the Hagedorn-Jensen method, urinary sugar by Cole's method and urinary ketones by the Van Slyke-Denigès method. Blood sugar estimations were usually carried out after a 12-hour fast.

Each pancreas was given double fixation in Helly-Zenker solution and cut in paraffin. Sections were stained by a modified hæmatoxylin and eosin method. The modification consisted in the interpolation of brief treatment with potash alum between two periods of staining with eosin. This, along with double fixation, gave good differential staining of the A- and B-cells in the islets. The number of islets was estimated by observing the degree of separation of the islets, the size of the islets and the proportion of islets consisting largely or wholly of A-cells. The size of the islets was gauged by the projection technique of Ogilvie (1937) and was based on the examination of 100 unselected samples.

Six rabbits (35, 36, 37, 42, 43 and 45) were made diabetic with alloxan. Five of the diabetic animals (35, 36, 37, 42 and 45) were then given anterior pituitary extract, while the remaining rabbit (43), a litter-mate of one of the treated animals (42), was retained as an untreated control.

RESULTS

Clinical data

Rabbit 35 (male) had a normal blood sugar of 107 mg. per 100 c.c. and with alloxan acquired a blood sugar of 427 mg. per 100 c.c. and glycosuria of 14 per cent.* The animal then received six courses of anterior pituitary extract at average intervals of 25 days. Each treatment lasted 10-15 days and the extract given daily was equivalent to 0·5 g. of anterior pituitary gland per kg. body weight. Three courses incidentally increased the diabetes, while two produced no concurrent change and one, the fifth, was accompanied by almost complete disappearance of the glycosuria. The augmented diabetes returned to its former level after the earlier courses, but the fifth treatment was followed by a sugar-free period of 3 days with a blood sugar of about 120 mg. per 100 c.c. The glycosuria then reappeared and rose to 14 per cent. The sixth course led to a blood sugar of 438 mg. per 100 c.c. and glycosuria of 19 per cent. The sugar-free period was characterised by a slightly reduced diet, an increased body weight and an augmented urinary volume.

Rabbit 36 (male) had a normal blood sugar of 119 mg. per 100 c.c. After alloxan it exhibited a very acute diabetes, necessitating temporary control by insulin. Later it recorded a blood sugar of 400 mg. per 100 c.c. and glycosuria of 27 per cent. The animal then received five courses of extract at average intervals of 23 days. Each course lasted 10-18 days and the daily dose was equivalent to 0·5 g. anterior lobe per kg. body weight. The five courses concurrently increased the diabetes, were never followed by improvement, and ended with a blood sugar of 497 mg. per 100 c.c. and glycosuria of 26 per cent.

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* This figure refers to the percentage of dietary polysaccharides excreted in the urine over a 24-hour period.

Rabbit 37 (male) had a normal blood sugar of 146 mg. per 100 c.c. and with alloxan developed a blood sugar of 400 mg. per 100 c.c. and glycosuria of 21 per cent. The animal then received five courses of extract at average intervals of 22 days. Each treatment was of 12 days' duration and consisted in the daily administration of 0·5 g. anterior lobe per kg. body weight. All the courses except the third were accompanied by increased glycosuria. The first treatment had no subsequent influence on the diabetes, but the next two courses together induced a blood sugar of 154 mg. per 100 c.c. and glycosuria of 1 per cent. The last two treatments were followed by a blood sugar of 373 mg. per 100 c.c. and glycosuria of 26 per cent. The reduction of the glycosuria to 1 per cent. was accompanied by normal diet, body weight and urinary volume.

Rabbit 42 (female) had a normal blood sugar of 167· mg. per 100 c.c. and after alloxan developed a blood sugar of 420 mg. per 100 c.c. and glycosuria of 26 per cent. (fig. 1). It then received five courses of extract starting on the 30th, 59th, 87th, 123rd and 309th days. Each treatment lasted 10 days except the fourth, which was for 3 days only, and the daily dose of anterior lobe varied between 0·125 and 0·5 g. per kg. body weight. Each of the five courses brought about an incidental increase of the diabetes. The first course of treatment was not followed by improvement, but the next two courses resulted on the 120th day in a blood sugar of 172 mg. per 100 c.c. and no glycosuria. The glycosuria, however, reappeared on the 121st day and slowly increased to 22 per cent. on the 278th day. The fifth course finally led to a blood sugar of 350 mg. per 100 c.c. and glycosuria of 30 per cent. The sugar-free period on the 120th day was accompanied by normal diet, body weight and urinary volume.

Rabbit 45 (male) had a normal blood sugar of 139 mg. per 100 c.c. and with alloxan acquired a blood sugar of 453 mg. per 100 c.c. and glycosuria of 28 per cent. (fig. 2). It then received twelve courses of extract at intervals of 10-70 days (average 30 days). Each treatment lasted 10-20 days and the daily amount of anterior lobe varied between 0·125 and 0·75 g. per kg. body weight. The twelve courses were divisible into two groups according to whether they incidentally had no influence upon or aggravated the disease. The first treatment had no effect on the diabetes, but the condition was afterwards modified by all except one of the other eleven courses. The modification consisted of an initial reduction in blood sugar, glycosuria and urinary volume and an increase in body weight, and a secondary change in the reverse order in each of these factors. The result was a fall in the glycosuria to 4 per cent. or less after nine of the courses and disappearance of sugar from the urine after four treatments. Three of the sugar-free periods were for one day only, but the fourth lasted 22 days. Each of the periods, moreover, was characterised by a normal blood sugar and urinary volume, a moderately reduced diet and a normal or increased body weight. On the 507th day the

animal had a blood sugar of 255 mg. per 100 c.c. and glycosuria of 11 per cent., but, if left, would probably have risen to much higher levels.
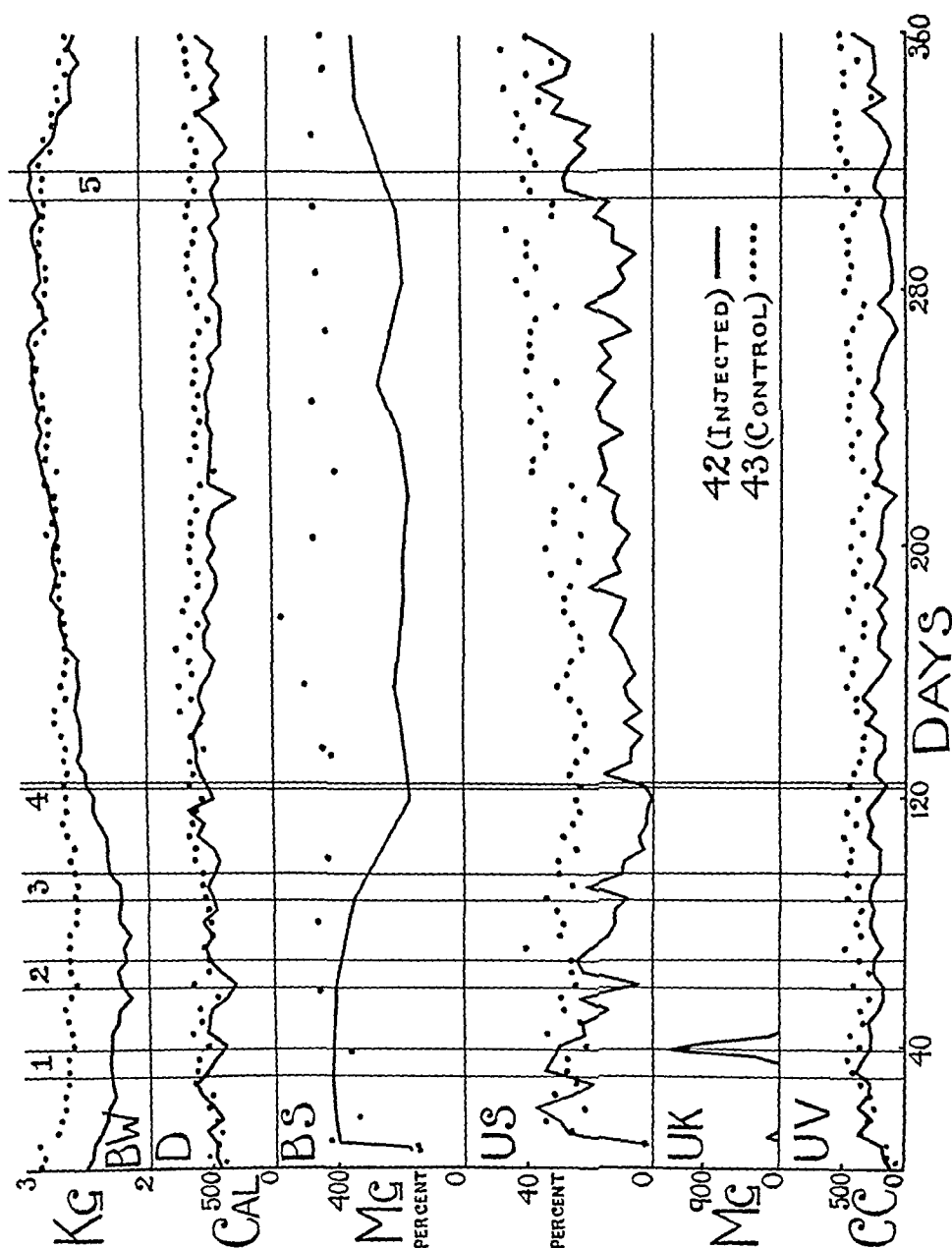
Rabbit 43 (female), a litter-mate of rabbit 42, was used as an untreated control. It had a normal blood sugar of 139 mg. per 100 c.c. and, after the same amount of alloxan per kg. body weight as was

given to rabbit 42, developed a blood sugar of 422 mg. per 100 c.c. and glycosuria of 28 per cent. (fig. 1). This state was maintained
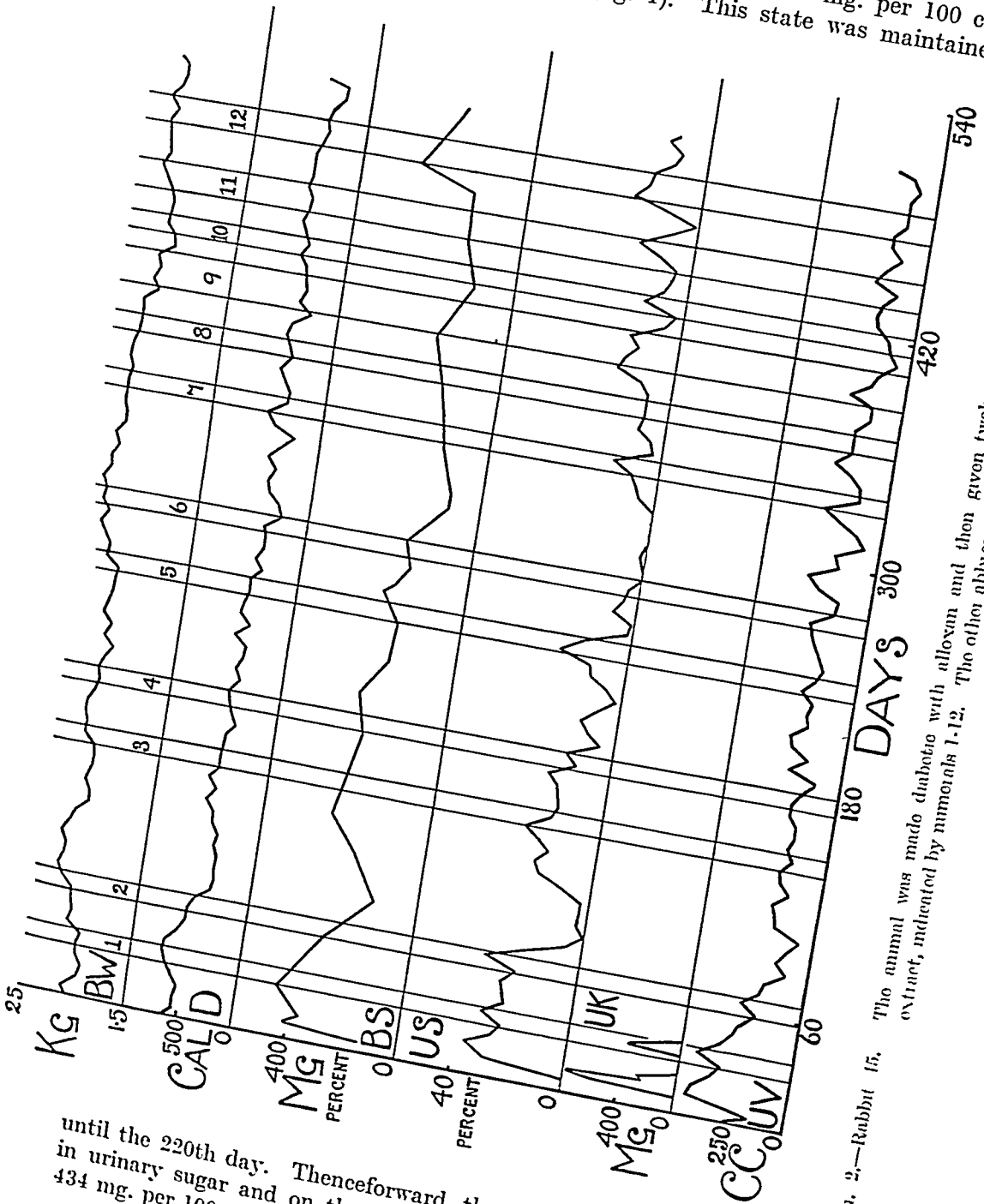
FIG. 2.—Rabbit 15. The animal was made diabetic with alloxan and then given twelve courses of anterior pituitary extract, indicated by numerals 1-12. The other abbreviations are as in fig. 1.

until the 220th day. Thenceforward, the animal showed an increase in urinary sugar and on the 360th day registered a blood sugar of 434 mg. per 100 c.c. and glycosuria of 40 per cent.

Histological data

These refer to the pancreatic islets and ducts as follows :—

1. *Number and size of islets.* Each animal of the series, particularly rabbit 36, showed a marked reduction in the number and size of its islets. No exact numerical estimate was made of the amount of diminution, but a measure of the reduction dimensionally is given for rabbits 42 and 43 in the section on regeneration (*vide infra*).

2. *Atrophy of islets to groups of A-cells.* This condition expressed itself in various stages. Thus some islets were made up of A- and B-cells in about equal proportions, while others consisted mostly or wholly of A-cells (fig. 5). The A-cells, moreover, having become relatively increased, tended to be localised in one or two distinct groups and, both in such collections and in purely A-cell islets, were usually arranged according to one or other of several patterns. One grouping showed a core of polyhedral elements enclosed by columnar cells with basally placed nuclei. Another consisted in a single row of columnar cells with the nuclei arranged along one side, while still others comprised two rows of columnar cells with the nuclei situated centrally or peripherally. The proportion of purely A-cell islets varied between 7 per cent. in rabbit 45 and 28 per cent. in rabbit 36, with an average of 18 per cent. The A-cells were histologically normal.

3. *Hydropic degeneration of B-cells.* Such damage was manifest in replacement of the cytoplasm and granules of the cells by serous fluid (fig. 6). Loss of this material often occurred during preparation, causing the cells to appear finely vacuolated or as a clear space with a nucleus and bounding membrane. Serous replacement, even when advanced, was not always accompanied by enlargement. The cells, however, were often swollen and occasionally reached some six times the normal average size. The nucleus in normal or slightly enlarged cells retained its usual position, but in markedly swollen cells was often pushed to one side. Hydropic change was observed in more than 90 per cent. of the B-cell islets in rabbits 35, 36, 37 and 43 and rather less than 50 per cent. in rabbits 42 and 45, being graded as moderately severe and relatively slight in the two groups respectively.

4. *Regeneration of islets.* This phenomenon was evidenced by enlargement, budding, and the formation of new islets from ducts.

(a) **Enlargement.** The average area of the islets in rabbit 42, in control rabbit 43 and as the mean of 10 normal rabbits was in the order of 0·62, 0·42, and 1·02 sq. cm. respectively (figs. 3 and 4). In other words, the islets of the treated animal, although still abnormally small, were on average 48 per cent. larger than those of the untreated rabbit.

(b) **Budding.** Islets sometimes attracted attention by reason of their irregular or even bizarre configuration (figs. 7-9). Thus they occasionally consisted of three or four small masses clustered together or arranged in a row and attached broadly or only at points. The

ALLOXAN DIABETES

FIG. 3.—The upper, middle and lower groups each represent 100 unselected islets taken respectively from the pancreas of untreated rabbit 43, treated rabbit 42 and a normal rabbit. The islets of rabbit 42, although smaller than normal, average more than those of rabbit 43. × 40.
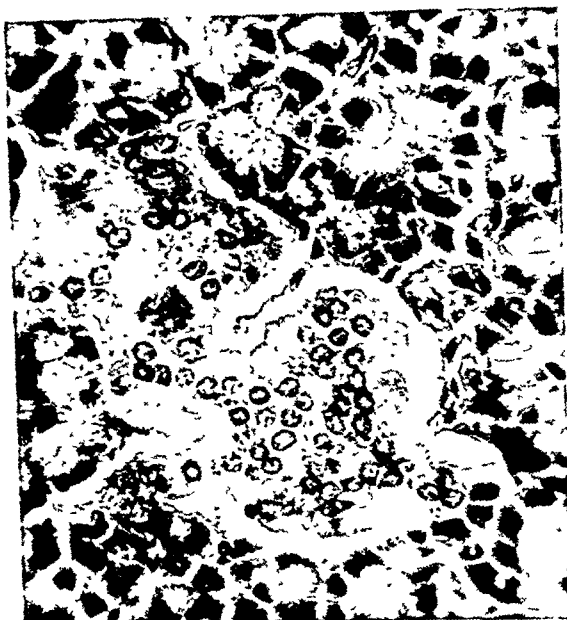
FIG. 5.—Rabbit 35. This pancreatic islet consists wholly of darkly stained A-cells and, as indicated by the irregular shape and numerous nuclei, probably shows regeneration. Hæmatoxylin and eosin. × 525.
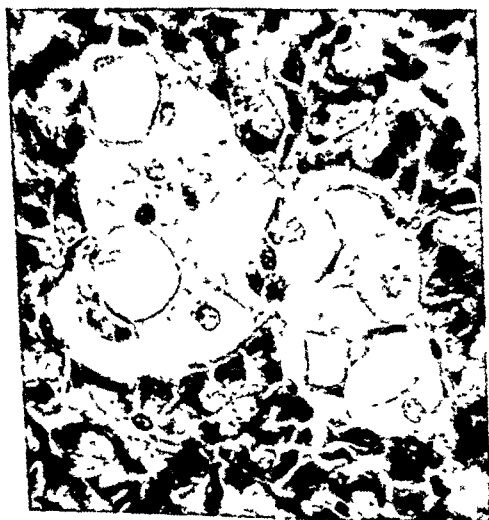
FIG. 6.—Rabbit 35. The islet shows hydropic degeneration of many B cells and a central group of darkly stained normal A-cells. H. and E. × 105.

FIG. 7.—Rabbit 45. The islet exhibits budding and early hydropic degeneration of occasional B cells. H. and E. × 200.

interpretation was that in these instances insular enlargement had taken place not so much circumferentially as by focal outgrowth. Such irregular islets mostly consisted of B-cells only or of a mixture of A- and B-cells. Purely A-cell islets were rarely abnormal in shape. Budding of B-cell islets with or without A-cells was observed in rabbits 35, 37, 42 and 45 to the extent of 5 or 6 per cent. of the B-cell islet population. The regenerating islets in rabbits 35 and 37, moreover, were respectively always or mostly hydropic, usually in moderate
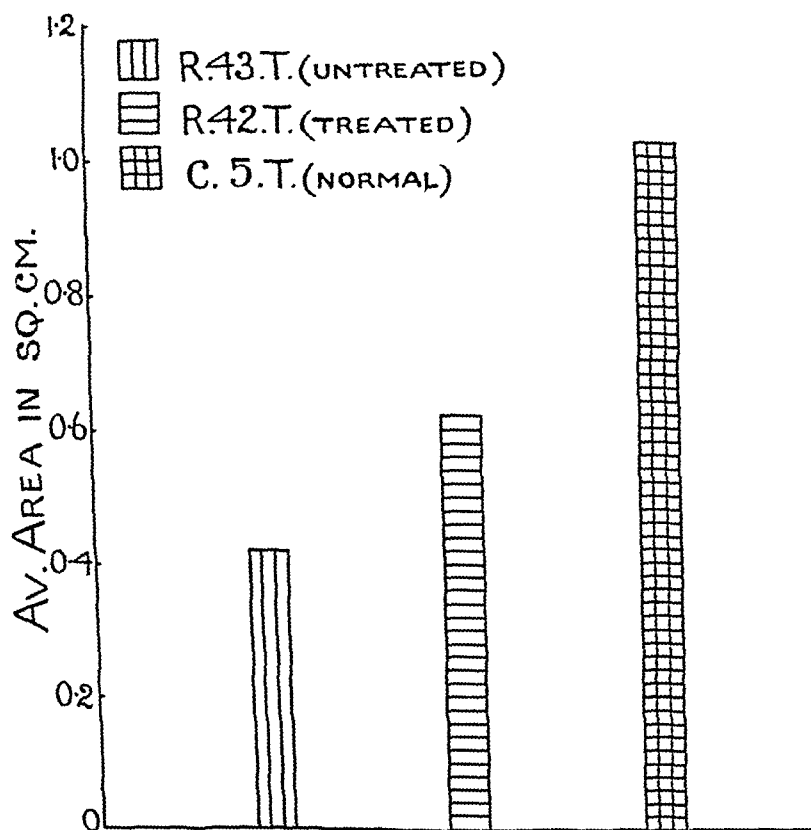
Fig. 4.—The columns indicate the average insular area in sq. cm. of the same three groups of 100 unselected islets as are shown in fig. 3. ×160.

or severe degree. On the other hand, the budding islets in rabbit 42 were normal and slightly or moderately hydropic in about equal proportions, while those in rabbit 45, although usually hydropic, were mostly so affected in only slight degree. Regenerating A-cell islets were very occasionally found in rabbits 35, 36, 37, 43 and 45.

(c) New islets from ducts. No related islets and ducts were observed in rabbits 36, 43 and 45, while rabbits 35, 37 and 42, in nearly the same amount of material, included respectively three, three and five examples of contiguous islets and ducts (figs. 10 and 11). Now the normal rabbit pancreas very rarely shows any apposed islets.

and ducts. Accordingly, a normal relation of islets and ducts was regarded as obtaining in rabbits 36, 43 and 45, whereas in rabbits 35, 37 and 42 the picture was interpreted as showing a growth of new islets from the ducts. These new units arose from the intralobular channels, had sometimes budded into other masses, and apparently consisted wholly of B-cells. They were hydropic in rabbits 35 and 37 but structurally normal in rabbit 42. No mitotic figures were found, either in enlarged pre-existing islets or in islets recently derived from ducts, and no local proliferation of the intralobular ducts was present in any pancreas.

5. *Hydropic degeneration of ducts.* This condition was characterised, as in the islets, by serous swelling of the lining epithelium with nuclear displacement (figs. 10 and 12). It was found in both the interlobular and intralobular ducts, principally the latter, affecting anything from single cells to long segments, and was either gradual or abrupt in its appearance. The condition was more marked in rabbits 35, 36, 37 and 43 than in rabbits 42 and 45.

DISCUSSION

Four of the five alloxan-diabetic rabbits (35, 37, 42 and 45), after varying amounts of treatment with anterior pituitary extract, showed evidence of improvement in their clinical state. This was striking in that marked hyperglycæmia and glycosuria were replaced by an almost normal or completely normal blood sugar and urine. The recovery, however, was transitory, and was followed by a reappearance and increase of the diabetic state. The administration of further extract did not subsequently influence the severity of the condition in rabbits 35, 37 and 42, whereas rabbit 45 continued to improve and regress after each of several more treatments. The four animals finally were—or would have become—as severely diabetic as they were before treatment with the extract.

Whether the temporary improvement of the four animals was spontaneous or due to the effects of the extract may be determined from the following facts. First, the litter-mate rabbits 42 and 43 were made equally diabetic with alloxan. Left untreated, rabbit 43 never recovered and indeed became progressively more diabetic. With extract, rabbit 42, on the other hand, improved to the extent of having no glycosuria for one day, thereafter remaining permanently less diabetic than rabbit 43. Second, the fact that rabbits 35, 37, 42 and 45, after becoming almost or completely sugar-free on one or more occasions, subsequently relapsed, indicates that the animals had an inherent tendency to remain diabetic and consequently exhibited no capacity for spontaneous recovery. Third, alloxan-diabetic rabbits having blood-sugar levels comparable with those of the present series were noted by Kennedy and Lukens to remain without improvement for so long as they were kept under observation up to a maximum

ALLOXAN DIABETES

FIG. 8.—Rabbit 37. The islet is characterised by budding and hydropic degeneration of a single B-cell (middle left). H. and E. ×270.
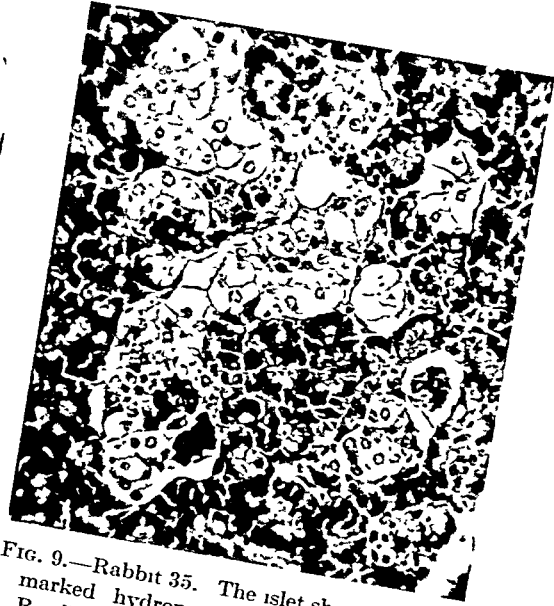
FIG. 9.—Rabbit 35. The islet shows budding, marked hydropic degeneration of many B-cells, and small groups of darkly stained normal A-cells. H. and E. ×180.
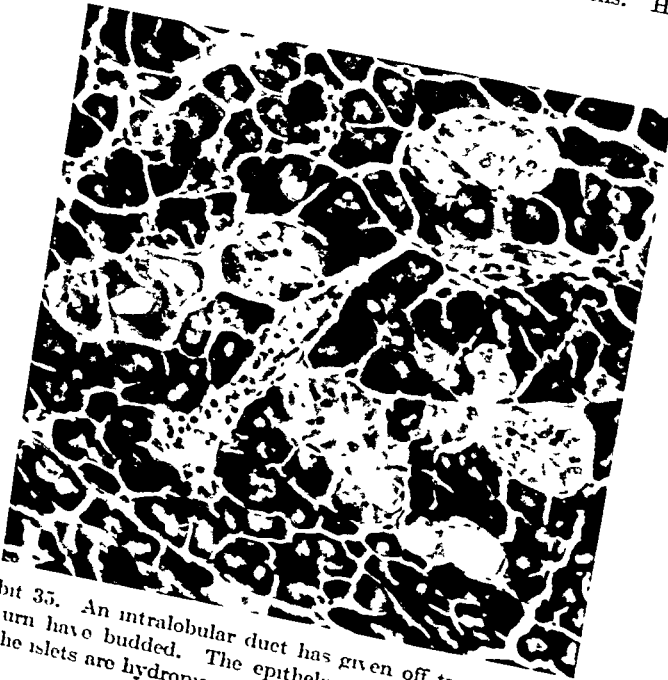
FIG. 10.—Rabbit 35. An intralobular duct has given off two (probably three) islets, which in turn have budded. The epithelium lining the duct and some of the B-cells in the islets are hydropic. H. and E. ×170.

of nine months. Fourth, the manner in which improvement followed soon after a given treatment was a feature of all four animals and particularly of rabbit 45, which promptly responded to each of 10 out of a total of 12 courses. Such a relationship obtaining in different animals and indeed often in the same rabbit could scarcely be fortuitous. On the basis of these facts, the transitory improvement which ensued in the four alloxan-diabetic rabbits with treatment may justifiably be regarded as an effect of the pituitary extract.

The probable mechanism whereby anterior pituitary extract temporarily improved four alloxan-treated animals is revealed in the difference between their pancreatic islets and those of the treated but unresponsive rabbit 36 and the untreated rabbit 43. All the rabbits showed degeneration of their islets, but regeneration of islet tissue was evident only in animals which recovered transitorily with treatment. The degenerative changes consisted of a reduction in the number and size of the islets, atrophy of the islets to groups of A-cells, and hydrops of the B-cells. The first two of these were immediate effects of the alloxan in destroying the B-cells, while the third is regarded as due to the prolonged hyperglycæmia (Kennedy and Lukens). Support is given to this last view by the present investigation in that the hydropic state of the B-cells was more marked in association with the persistently higher glycæmias. Regeneration of the islets as indicated by marginal budding and suggestive new growth from the ducts was confirmed by the islets being on the average larger in treated rabbit 42 than in its untreated litter-mate 43. A similar enlargement of the islets following treatment with pituitary extract was observed in rats by Richardson and Young (1937-38) and in normal rabbits by Ogilvie (1944, 1944-46). who in one animal also noted (1944) proliferation of the small pancreatic ducts and differentiation of new islets therefrom. Further, Marks and Young (1939, 1940) have shown that the treatment with extract, which in rats doubles the amount of islet tissue, also produces twice the quantity of extractable insulin. Such effects on the pancreatic ducts and islets and on the insulin content have been attributed to a pancreatropic factor in the extract (Ogilvie, 1944). This factor. by increasing the islet tissue and available insulin, may consequently be regarded as responsible for the temporary recovery, in the present work, of four of the five treated alloxan-diabetic rabbits.

The improvement of the four rabbits ensued despite the more severe diabetes accompanying the administration of extract. The explanation is that the augmented condition during treatment was due to the prepared diabetogenic influence of the extract, while the pancreatropic factor, acting through the above mechanism, was naturally longer in coming into play and then was aided by the diabetogenic action having ceased to operate. On the other hand. the reason for the failure of rabbit 36 to improve with treatment undoubtedly lay in its islet tissue having been so depleted by the

alloxan treatment as to be overstrained by the diabetogenic influence and consequently incapable of responding to the pancreatropic action of the extract. Finally, the temporary character of their recovery points to the limited sensitivity of the four responsive animals to the pancreatropic factor, while the way in which three of them failed to respond beneficially to treatment after temporary improvement suggests an actual loss of sensitivity to its action.

The present investigation is a corollary to the administration of alloxan to animals which have been (or were subsequently) subjected to hypophysectomy. Such experiments have been carried out by Duff and Starr (Duff, 1945), Gaarenstroom (1946-48), and Bailey *et al.* (1947), and indicate that alloxan has then little or no diabetogenic effect. The suppression of diabetes in the hypophysectomised-alloxanised animal, of course, is comparable with the ameliorating effect of hypophysectomy in animals made diabetic by pancreatectomy (Houssay and Biasotti, 1930). These findings imply, and receive support from the fact, that anterior pituitary extract given to the present alloxan-diabetic animals often increased their diabetes. The temporarily beneficial effect of the extract in four animals, however, could not have been adduced from these experiments and has here been attributed to the limited sensitivity of the rabbit to the pancreatropic action of the extract.

SUMMARY

1. Six rabbits were made severely diabetic with alloxan. Five of them were then treated with crude anterior pituitary extract, while the sixth—a litter-mate of one of the treated animals—was kept as an untreated control.

2. Four of the treated rabbits improved markedly. Recovery, however, was but temporary, and during the succeeding diabetes further extract did not influence the condition in three of the animals. The fourth continued to show improvement and regression after each of several additional courses of treatment.

3. Finally the five treated animals were, or obviously would have become, as severely diabetic as before treatment with extract. The control rabbit did not recover.

4. The pancreatic islets of both treated and control rabbits showed a reduction in number and size, atrophy to groups of A-cells and hydropic degeneration of B-cells, while the islets of the four rabbits which recovered temporarily with extract exhibited regeneration as well, as evidenced by enlargement and budding of the islets and a suggestive growth of new islets from the ducts.

5. Hydropic degeneration of the B-cells was more marked in association with the persistently higher glycæmias.

6. Hydropic degeneration was noted in the small pancreatic ducts of all the diabetic animals, whether treated or not.

Alloxan Diabetes
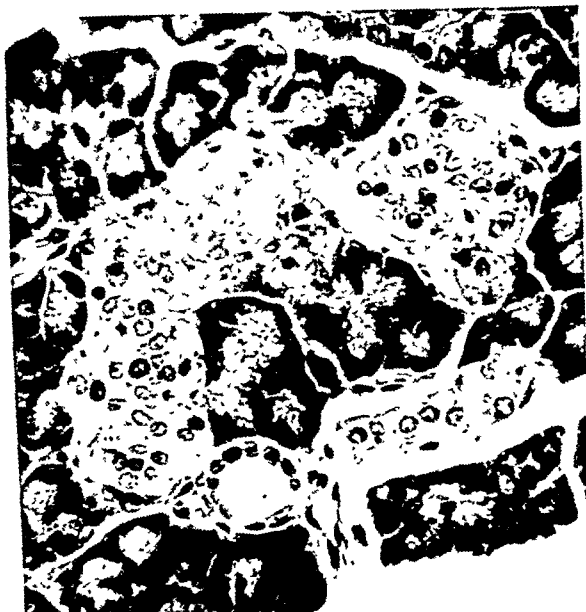
Fig. 11.—Rabbit 42. An intralobular duct has budded islet tissue on either side so as to form an almost complete circle. H. and E. ×375.
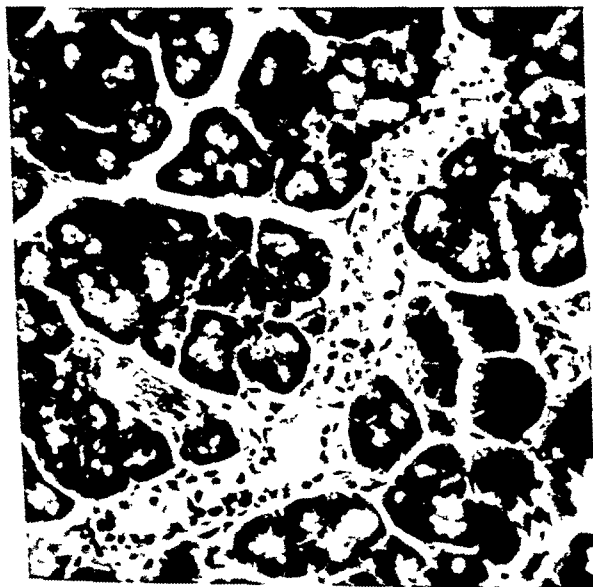
Fig. 12.—Rabbit 35. An interlobular duct shows hydropic degeneration of its epithelium. H. and E. ×225.

7. The temporary improvement of the four treated alloxan-diabetic rabbits is attributed to the pancreatropic action of the pituitary extract.

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AN ANALYSIS OF THE EFFECTS OF ββ'DICHLORO-DIETHYLMETHYLAMINE HYDROCHLORIDE AND OF FORMALIN ON THE WEIGHT OF THE LYMPHOID ORGANS OF INTACT AND ADRENAL-ECTOMISED MICE

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THE great destructive and depressant effect of ββ'dichlorodiethylmethylamine hydrochloride (nitrogen mustard) on the bone marrow and lymphoid organs has been established by numerous experimental investigations (Gilman and Philips, 1946 ; Kindred, 1947 ; Cameron *et al.*, 1947 ; Graef *et al.*, 1948) as well as by clinical trials in man (Rhoads, 1946 ; Wilkinson and Fletcher, 1947). The effects of this vesicant include widespread cellular destruction, usually with pyknosis, in the hæmopoietic tissues, accompanied by prominence of large phagocytes which are active in clearing away cellular debris. The rate of mitosis is decreased (Kindred). There is also a fall in the output of lymphocytes from the thoracic duct and a decreased production of neutrophils in the bone marrow (Cameron *et al.*), resulting in lymphocytopenia and neutropenia in the circulating blood.

Kindred has drawn attention to the resemblance of the lymphocytopenia and degenerative changes produced by the nitrogen mustards in the lymphoid organs to those produced by the non-specific agents which cause the " alarm reaction " of Selye (Selye, 1936*a* and *b*, 1937, 1946) and to those reported to follow injections of pituitary adrenotropic hormone and adrenal cortical extracts (Dougherty and White, 1945). Evidence has been produced by Selye (1936*a* and *b*) and Leblond and Segal (1938 *a* and *b*) that the degenerative changes in the lymphoid organs in the alarm reaction are considerably less marked in adrenalectomised animals. Adrenalectomy also prevents involution of the lymphoid organs of animals treated with pituitary adrenotropic hormone (Simpson *et al.*, 1943 ; Dougherty and White, 1943). In the case of animals treated with nitrogen mustard there is evidence of increased activity (enlargement and depletion of lipoid) of the adrenal cortex (Karnofsky and Graef, 1942-43, unpublished data quoted by Kindred ; Ludewig and Chanutin, 1946 ; Kindred, 1947). Karnofsky, Graef and Smith (1946) found, however, that the same degenerative changes occurred in the lymphoid organs of adrenalectomised animals poisoned with ββ'dichlorodiethylmethylamine hydrochloride as occurred in the non-adrenalectomised controls, although the effect was possibly less severe. Kindred also concluded that the nitrogen mustards produced at least part of their effect on the lymphoid organs by direct action not mediated through the adrenals.

The results of quantitative experiments on lymphoid tissue must be evaluated with considerable care, as the factors which influence its amount are numerous and complex. The state of nutrition of the animal, for example, is of great importance, the lymphoid organs becoming very small in starved animals.

The thymus is especially sensitive to inanition (Hammar, 1905; Jonson, 1909; Levin, 1912; Jackson, 1915; Stewart, 1916; Andreasen, 1943; Reinhardt, 1943). The lymph nodes, though less sensitive to starvation than the thymus, also quickly diminish in size (Hellman, 1913-14; Jolly, 1914; Jackson, 1925; Andreasen, 1943); so also does the spleen (Hellman, 1925-26; Andreasen, 1943). Certain vitamin deficiencies are also known to cause regression of lymphoid organs (Cramer *et al.*, 1921; Uotila and Simola, 1938; Stoerk, 1946).

The amount of lymphoid tissue also varies with the age of the animal. This question has been exhaustively investigated by Hellman (1913-14) in the rabbit, and reduction in size of the lymph nodes with age was known by the older anatomists to occur in man (von Haller, 1778; Bichat, 1801: both cited by Hellman, 1913-14). The proportion of splenic lymphatic tissue in man has also been found to vary with age (Hwang *et al.*, 1938).

Endocrine influences are of considerable importance in relation to the weight of the lymphoid organs. The thymus becomes smaller during pregnancy (Calzolari, 1898-99; Henderson, 1904; Goodall, 1904-05; Gellin, 1909-10; Persike, 1940) and under the influence of pituitary or gonadal sex hormones (Chiodi, 1940; Persike, 1940; Ross and Korenchevsky, 1941; Reinhardt and Wainman, 1942). Adrenalectomised animals manifest enlargement of the thymus (Jaffé, 1924; Reinhardt and Holmes, 1940; Grégoire, 1943), while intact animals injected with pituitary adrenotropic hormone show thymic involution (Ingle, 1938; Evans *et al.*, 1938; Dougherty and White, 1943; Simpson *et al.*, 1943), as do intact or adrenalectomised animals injected with adrenal cortical extracts (Ingle, 1938; Ingle and Mason, 1938; Wells and Kendall, 1940; Heilman and Kendall, 1944).

On the other hand there is experimental evidence (Utterström, 1910; Courrier, 1921; Chiodi, 1940; Reinhardt and Wainman, 1942) that the thyroid hormone opposes, though not powerfully, the depressant action on the lymphoid organs exerted by the gonadal and adrenal cortical steroids. This confirms the experience of students of human disease, who have reported the frequent finding of enlargement of the thymus (and lymph nodes) at autopsy in persons dying with hyperthyroidism.

Lymphoid tissues other than the thymus respond in a similar manner, though less notably, to the administration of hormones. Thus oestrogens cause involution of lymph nodes (Cramer and Horning, 1939; Chiodi, 1940; Reinhardt and Wainman, 1942), as usually does adrenal cortical extract (Dougherty and White, 1943). However, in some experiments reported by Yoffey *et al.* (1946) it was found that prolonged administration of an adrenal cortical preparation in the rat caused hyperplasia of the lymph nodes, although the same preparation in the cat caused a fall in the number of lymphocytes issuing from the thoracic duct. Another unusual finding (Dmochowski and Horning, 1947) was that prolonged painting of the skin of male mice of two inbred strains with 0·01 per cent. ketohydroxyœstrone in chloroform produced, in a small proportion of the animals, considerable hyperplasia of the thymus and lymph nodes, with evidence of local invasiveness. However, in many of the mice which did not manifest this type of hyperplasia the thymus was smaller than normal.

The present investigation was undertaken to study in more detail the effect of adrenalectomy on the involution of the lymphoid tissues in animals poisoned with $\beta\beta'$dichlorodiethylmethylamine hydrochloride, contrasted with the effect of formalin. Subsidiary objects of the work were to investigate the relationship of the response of the individual lymphoid organs to that of the lymphoid tissue as a whole, to determine whether any sex differences were demonstrable and

whether different responses occurred in animals subjected to identical experimental procedures but on successive occasions.

Two experiments were performed. In expt. 1 the effect of the nitrogen mustard was studied, in expt. 2 the effect of formalin.

Experiment 1

Sixty mice of a stock inbred laboratory strain were used. They were aged 7 weeks, and half were males and half females. The mean weight was 21.4 ± 0.2 g. They were selected in 12 batches (subgroups) of 5 animals of the same sex from the larger stock batches of 10-12 animals by choosing those in which the body weight most nearly agreed with an arbitrarily fixed value (21.0 g.). In this laboratory batches of animals are made up by combining 2 or 3 litters of weanlings as soon as they are removed from the mothers. The animals selected were given an intraperitoneal injection of 0.1 ml. of 0.5 per cent. pontamine blue to mark the lymphoid organs. The 12 batches or subgroups, each of 5 animals, were then allotted at random to the 12 subgroups of the experimental plan. This plan is shown in table I. In it are set out the subgroup means, each with its standard error, of the crude experimental data.

On the following day the appropriate 20 animals, comprising 2 subgroups of males and 2 of females, the 4 subgroups together constituting a " series ", were anæsthetised with ether, the skin of the back was shaved and swabbed with 70 per cent. alcohol, and both adrenals were removed with small ring forceps through lumbar incisions which were then closed with interrupted silk sutures. A further series of 4 subgroups served as " sham-operated " controls. In these animals the operative procedure was identical, the peritoneal cavity being entered on both sides, but the adrenals were not removed. The remaining series of 20 animals served as normal controls.

After operation the animals were fed on standard rat cake, with unlimited drinking water. The adrenalectomised animals were given 1 per cent. NaCl to drink instead of plain water.

On the day after operation half the mice from each series, 30 in all, were injected subcutaneously with $\beta\beta'$dichlorodiethylmethylamine hydrochloride in a dose of 1 mg. per kg. Four animals, one from each of the subgroups of the adrenalectomised series, died during the following three days. These animals were discarded from the experiment. The 56 survivors were killed with coal gas after an experimental period of 72 hours from the time of injection of the vesicant. The abdomen and thorax of each mouse was opened and the skin reflected from the ventral surface to expose the superficial lymph nodes—cervical, axillary and inguinal. The animal was then pinned out on a card and fixed whole in 10 per cent. aqueous formalin for at least 7 days.

Using a binocular dissecting microscope ($\times 7$) all the lymph nodes were dissected out and re-fixed in groups in fresh formalin. Superficial cervical, deep cervical, axillary, inguinal, mediastinal, renal, mesenteric, para-aortic and iliac groups were identified, carefully freed from fat and fascia and removed. The nodes were found to be remarkably constant in position, number and size—even in shape—from animal to animal. The previous injection of pontamine blue facilitated the identification of even very small nodes. At the same time the thymus and spleen were removed and all the Peyer's patches, the latter by a circumferential incision as close as possible to the margin of the lymphoid nodule. The peritoneum and mucosa were left attached to the patch. It was not possible to remove every one of the solitary lymphoid follicles of the

colon, as many of them were very small. The largest of these follicles, however, were removed in the same fashion as the Peyer's patches.

After re-fixation in fresh formalin all tissues were lightly blotted to remove external fluid, and weighed on a small torsion balance to the nearest mg. The weighings were all carried out by one person, and in as uniform a manner as possible. After being weighed, the organs were returned to formalin and prepared for histological study.

TABLE I

Plan of expt. 1. Means of subgroups (5 animals per subgroup, except in adrenalectomised series)

| | | Injected with N-mustard | | Not injected | | Standard error |
		Males	Females	Males	Females	
Normal	a	21·48	20·50	19·84	22·06	0·53
	b	22·66	20·92	22·20	23·18	0·58
	c	75·2 .	90·2	100·8	105·6	8·0
	d	15·4	15·4	31·6	27·0	3·10
	e	189·0	181·0	266·6	213·6	20·8
	f	104·2	92·8	114·6	65·0	12·9
	g	285·2	334·0	356·4	327·6	26·2
Sham-operated	a	19·66	20·56	20·70	20·98	0·53
	b	21·74	20·56	21·22	21·80	0·58
	c	79·8	55·6	72·2	97·0	8·0
	d	13·8	13·4	24·8	30·4	3·10
	e	159·8	149·2	136·4	193·4	20·8
	f	74·6	56·0	51·2	67·2	12·9
	g	252·2	209·2	243·0	300·8	26·2
Adrenal-ectomised *	a	20·48	19·86	21·46	21·46	0·59
	b	20·45	20·30	20·65	20·15	0·64
	c	83·0	60·25	76·0	87·25	9·0
	d	21·25	23·5	31·75	37·75	3·5
	e	183·75	137·75	203·5	229·75	23·2
	f	93·25	59·25	89·75	60·75	14·4
	g	287·0	213·75	268·75	295·25	29·3

a. Initial body weight (g.).
b. Final body weight (g.).
c. Superficial lymph nodes (mg.).
d. Thymus (mg.).
e. Gross weight of spleen (mg.).
f. Estimated weight of splenic lymphoid tissue (mg.).
g. Estimated weight of total lymphoid tissue (mg.).

* Four animals per subgroup.

An approximate estimate of the proportion of the total splenic weight which consisted of lymphoid tissue was made by projecting with a lantern the image of a stained median longitudinal section of the organ on paper, tracing the outline of the whole section and of the contained lymphoid nodules, and subsequently estimating by planimetry the proportion of the whole area made up of lymphoid areas. The gross weight of the spleen was then multiplied by this fraction.

A similar estimate of the weight of the lymphoid tissue in the Peyer's patches, which were weighed with the mucosa and peritoneum attached, was made by dividing the weight of the composite tissue by two, as preliminary experiments

by projection and planimetry had shown that approximately half of the tissue was made up of lymphoid tissue.

By these means an estimate was obtained of the total weight of the lymphoid tissue, excluding the thymus, after its fixation in formalin.

Experiment 2

In this experiment 96 mice, 48 of each sex, were used. They were of the same strain as those used in expt. 1. Their ages ranged from 8 to 10 weeks, but those used in each of the three stages were of the same age ; approximately 8 weeks in stage (*a*), 9 weeks in stage (*b*) and 10 weeks in stage (*c*). The plan of this experiment is shown in table II. The experiment was performed in three stages at weekly intervals, for two reasons :—(i) the large number of animals employed made a single stage experiment impracticable ; (ii) information was desired as to whether significant differences existed between the weights of the lymphoid organs of different batches of animals from the same strain, when subjected to identical experimental procedures but on successive occasions.

In expt. 2, as in expt. 1, there were two "treatments"—"injected with formalin" and "not injected". The dose of formalin used was 0·4 ml. of 10 per cent. aqueous solution per 100 g. body weight. This was given once daily, subcutaneously, on each of three successive days.

There were 4 series, each of 24 animals, as under. (1) *Normal.* (2) *Anœsthetised.* These animals were anæsthetised with ether for 15 minutes, this being the approximate period of anæsthesia occupied by the operative procedures in the other two series. This series was used to study any effect of the anæsthesia as such on the weight of the lymphoid organs. (3) *Sham-operated.* These animals were anæsthetised with ether, the skin of the back shaved and painted with acriflavine and the abdomen opened on both sides in the lumbar region. The adrenals were not removed. (4) *Adrenalectomised.* In these animals the adrenals were removed by the method already described. One ml. of 5 per cent. dextrose in 0·9 per cent. NaCl containing 400 units of crystalline sodium penicillin per ml. was introduced into the peritoneal cavity of each of the sham-operated and adrenalectomised animals. It was found that this infusion was of very considerable benefit in the adrenalectomised animals, maintaining them in better condition than in expt. 1 in which it was not used. For the adrenalectomised mice 5 per cent. glucose-saline was provided for drinking during the first post-operative day ; thereafter 1 per cent. saline without glucose. The diet for all animals was standard rat cake as in expt. 1.

In each stage, as shown in table II, 32 animals, 16 of each sex, were used. All the mice were of approximately equal body weight, and pairs were allotted at random to the "series", "treatment" and "sex" subgroups of the experimental plan. On the first day the animals were each injected with 0·1 ml. of 0·5 per cent. pontamine blue to mark the lymphoid organs. On the second day the operations and the administration of ether were carried out. On the morning of the third day injections of formalin were begun and repeated on the two subsequent days. On the afternoon of the fifth day, seven hours after the third and last injection of formalin, the animals were killed with coal gas, the abdomen and thorax of each opened, and the skin reflected from the ventral surface to expose the superficial lymph nodes. The animals were then pinned out on cards as in expt. 1 and fixed whole in 10 per cent. aqueous formalin for at least 7 days.

The second and third stages of the experiment were carried out in the same way. The lymphoid organs were dissected out and weighed on the torsion balance as in expt. 1. Two animals died during the course of expt. 2 : both had been adrenalectomised. To facilitate the computations, estimated values

were inserted for the numerical data that would have been furnished by these animals. Two degrees of freedom were deducted to allow for the bias introduced into the analysis of variance by the missing values.

TABLE II

Estimated total lymphoid tissue (mg.) of animals of expt. 2

	Stage	Injected with formalin		Not injected	
		Males	Females	Males	Females
Normal . .	*a*	327	209	289	276
		303	289	277	190
	b	248	180	387	250
		167	211	259	241
	c	206	238	244	253
		170	190	274	247
Anæsthetised .	*a*	275	225	267	293
		343	236	351	212
	b	164	321	327	197
		212	229	329	172
	c	221	236	265	221
		188	187	260	269
Sham-operated .	*a*	341	229	397	242
		165	210	243	202
	b	219	142	330	307
		194	171	396	143
	c	135	174	168	184
		167	119	187	184
Adrenalectomised .	*a*	(378) *	303	406	279
		378	218	307	212
	b	409	312	341	251
		460	285	263	201
	c	278	253	276	302
		232	241	281	(302*)

* Estimated value.

ANALYSIS OF RESULTS

The full numerical results obtained are shown in tables which have been deposited with the librarian, General Library, British Museum (Natural History), London, S.W.7. Some averages derived from these data are shown in tables I and III.

The series of analyses of variance of the weights of the component organs of the lymphoid system and of the total lymphoid tissue are not shown *in extenso* for reasons of space. However, the method of separating the total sum of squares for each experiment and some of the values obtained for the mean squares are shown in tables IV and V.

Experiment 1

In this experiment there was a suggestive difference in initial body weight between the treated and untreated groups, although *a priori* it was expected that the initial body weights would form a homogeneous population. The difference consisted largely in a

TABLE III

Expt. 2. Means of groups (3 stages combined, i.e. 6 animals per group)

		Injected with formalin		Not injected		Standard error
		Males	Females	Males	Females	
Normal	a	20·77	19·87	20·77	20·01	0·60
	b	90·35	68·80	92·35	68·64	7·1
	c	19·21	21·0	27·83	36·0	2·8
	d	149·83	116·33	182·5	146·33	21·3
	e	236·83	202·83	288·33	242·83	19·4
Anæsthetised	a	20·56	20·06	21·33	19·89	0·60
	b	98·53	69·64	122·56	71·14	7·1
	c	19·33	22·2	24·0	35·0	2·8
	d	133·0	122·33	185·5	136·83	21·3
	e	233·83	239·0	316·5	227·33	19·4
Sham-operated	a	19·62	19·36	21·46	20·22	0·60
	b	79·00	49·10	89·18	57·28	7·1
	c	10·18	13·33	22·33	17·0	2·8
	d	128·0	117·33	212·5	141·66	21·3
	e	203·5	174·16	286·83	210·33	19·4
Adrenal-ectomised	a	22·31	19·71	20·67	18·92	0·60
	b	102·87	84·50	106·21	90·35	7·1
	c	29·83	31·0	25·0	25·33	2·8
	d	239·33	193·83	206·16	210·5	21·3
	e	315·33	268·66	312·33	249·0	19·4

a. Initial body weight (g.).
b. Superficial lymph nodes (mg.).
c. Thymus (mg.).
d. Spleen (mg.).
e. Total lymphoid tissue (mg.).

TABLE IV

Values and significance of mean squares in expt. 1

	Degrees of freedom	Initial body weight (g.)	Final body weight (g)	Superficial lymph nodes (mg.)	Thymus (mg.)	Gross weight of spleen (mg.)	Splenic lymphoid fraction (mg.)	Total lymphoid tissue (mg.)
Total	55							
Subgroups	11	166·47	223·43	499·26	99·67	3132·4	1080·9	4702·7
Treatments (T)	2	285·95*	455·81†	1125·42†	307·0†	7040·2†	2071·6†	9726·1†
Series (S)	1	652·7*	292·6	3650·6†	2551·5†	21567·8†	434·6	24153·0•
"Operated" v. "Non-operated"	1	127·9	1530·1†	1770·1†	302·36†	13864·4†	5122·4†	29142·0†
Between "operated"	1	..	2270·3†	3538·3†	41·15	20451·6†	8624·7†	58032·1†
Sexes (B)	1	134·4	789·6*	2·1	563·56†	7277·1	1620·0	251·9
Interactions	7							
T.S.	1	300·4	157·8	56·0	23·1	1233·8	5501·8*	29·9
T.B.	2	223·3	88·7	124·35	3·56	3351·2	900·9	877·8
S.B.	1	427·3	787·5	1944·7*	8·7	2369·4	...	2076·6
T.S.B.	2	139·9	0·9	283·4	52·7	3112·8	...	653·1
	2	474·4	268·4	1171·3*	38·9	5507·3	...	10146·2
Within subgroups (error)	44	139·1	165·34	322·95	47·83	2155·5	833·3	3446·1

* Mean square significant between 5 per cent. and 1 per cent.
† Mean square significant beyond 1 per cent.

greater initial body weight among the untreated females (mean 21·59g.±0·32 g.) as compared with the treated females (20·40±0·32 g.)

TABLE V

Expt. 2.   Values and significance of the mean squares

	Degrees of freedom	Initial body weight (g.)	Superficial lymph nodes (mg.)	Thymus (mg.)	Spleen (mg.)	Total lymphoid tissue (mg.)
Total	93					
Subgroups	47	599 2†	1418 0†	165 34†	7452 8†	7588 85†
Stages (L)	2	3386 8†	6789 1†	51 3	61385 7†	26026 9†
Treatments (T)	1	38 8	1127 5	934 3†	16986 7*	17280 7†
Series (S)	3		3482 4†	682 4†	25424 9†	22938 2†
Sexes (B)	1	3348 9†	18343 0†	213 1*	22662 7†	57232 7†
Interactions	40	417 1†	5788 1†	114 3†		4032 4*
E × T	2	443 7		231 25*		
E × S	6		451 1			
E × B	2	1931 4†	2706 4†	112 9	10248 7*	16248 3†
T × S	3	6(2 4*		346 3†		9409 3*
T × B	1					7597 0
S × B	3		60† 1			
L × T × S	6		1115 1†			
E × T × B	2			98 49*		
E × S × B	6		551 −			
T × S × B	3					
E × T × S × B	6					
Within subgroups (error)	46	220 36	299 1	47 88	2727 25	2251 67

* Mean square significant between 5 per cent. and 1 per cent.
† Mean square significant beyond 1 per cent.

and with both treated (20·61±0·32 g.) and untreated (20·73±0·32 g.) males. It was therefore necessary to exercise great caution in interpreting any sex differences in the results of this experiment.

The analysis of variance of the final body weights showed highly significant differences between subgroups and series. The treatment difference was not significant. The effect of the experiment on the series means was further examined by separating the series sum of squares, with 2 degrees of freedom, into two parts, each with one degree of freedom ; *i.e.* " operated *v.* non-operated " and " between operated ". Both of these comparisons showed significant differences, but the former was significant beyond the 0·1 per cent. level, while the latter fell between 5 per cent. and 1 per cent. only. The effect of the operations was thus to cause three different populations of final body weights to appear. The normal control animals were heavier than the sham-operated, and these in turn were heavier than the adrenalectomised animals.

The " treatment × sex " interaction yielded a value of F which was significant between 5 per cent. and 1 per cent. The sex differences in initial body weight made this finding of little value. However only the females lost significant amounts of weight as a result of the injection of $\beta\beta'$dichlorodiethylmethylamine hydrochloride.

For the superficial lymph nodes (pooled cervical, axillary and inguinal groups) there were highly significant differences between treatments and series. The vesicant thus produced a real reduction in the weight of this group of nodes. Further light on the series difference was shed by separating the series sum of squares 3540·2

into two parts as before. It then appeared that no less than 3538·3 of this sum of squares was taken up by the comparison " operated *v.* non-operated ", leaving only 2·1 for the " between operated " comparison. Study of the series means made this clear. The superficial lymph nodes of the normal animals had a mean weight of 92·95± 4·0 mg., while the mean weight in the sham-operated animals was 76·15±4·0 mg., and in the adrenalectomised animals 76·6±4·5 mg.

The thymus also showed highly significant differences between both treatments and series. The treatment effect, in fact, was significant well beyond the 0·1 per cent. point, indicating that the vesicant had produced a very marked reduction in the weight of this organ. Separation of the series sum of squares in the same fashion as for the final body weight and the superficial lymph nodes showed, however, that the greater part of this sum of squares in the analysis of the thymic weights was made up of the quantity 563·56 for the " between operated " comparison, and that the sum of squares remaining for the " operated *v.* non-operated " comparison, 41·45, was not significant. In fact, if the series sum of squares was separated in another fashion, it then appeared that the comparison " adrenalectomised *v.* the rest " took up 574·1, with an F value of 12·00, leaving only 30·65 for the comparison " between the rest." Examination of the series means showed that the adrenalectomised animals had significantly larger thymuses (mean 28·6±1·5 mg.) than the sham-operated (20·6±1·5 mg.) and normal (22·4±1·5 mg.) animals. This finding is of interest when compared with the behaviour of the weights of the superficial lymph nodes, in which no difference in weight between the sham-operated and adrenalectomised animals was detectable.

Analysis of the gross weight of the spleen showed that there was a highly significant reduction as a result of the injection of $\beta\beta'$di-chlorodiethylmethylamine hydrochloride. A large series difference was also present, which, on separation into its two components showed that the behaviour of the gross splenic weight resembled that of the weight of the superficial lymph nodes and differed from that of the thymus in that the greater part of the sum of squares was accounted for by the " operated *v.* non-operated " difference. A sex difference was also present, as the females showed a significant reduction in splenic weight between the treated (156·9±8·8 mg.) and untreated (211·0±8·8 mg.) groups.

A similar series difference was present in the analysis of the splenic lymphoid tissue. The operated animals differed significantly from the non-operated. A suggestive sex difference was also present, in that the females had less splenic lymphoid tissue than the males. The treatment difference was however not significant, being, if present, obscured by the large errors of estimation.

The figure for total lymphoid tissue, calculated by addition of the weights of the lymphoid tissue in the component organs as already

described, was found to behave in a manner similar to the superficial lymph nodes. The treatment difference fell just short of the 1 per cent. point, and there was a large series effect which was mostly due to the difference " operated v. non-operated ". The analysis of the behaviour of the weight of the superficial lymph nodes therefore in this experiment accurately reflected the behaviour of the total lymphoid tissue.

From this series of analyses it was concluded that the injection of $\beta\beta'$dichlorodiethylmethylamine hydrochloride produced a reduction in the weight of the lymphoid organs, the thymus and the total lymphoid tissue. This result was expected on the basis of work previously reported in the literature. The effect of adrenalectomy on the response of the lymphoid tissues was more interesting. With respect to the superficial lymph nodes, spleen and splenic lymphoid fraction, as well as the total lymphoid tissue, the effect of operation alone—without adrenalectomy—was to reduce the amount of lymphoid tissue. It was considered that this effect was due at least in part to post-operative inanition, although post-operative adrenal cortical stimulation was probably also of importance. Adrenalectomy did not produce any more or any less reduction in the amount of lymphoid tissue than occurred in the sham-operated animals. In the case of the thymus, however, the weight of the organ was definitely greater in the adrenalectomised than in the normal or sham-operated animals. In respect of all the lymphoid organs there was a greater reduction of lymphoid tissue in the females than in the males. Adrenalectomy did not prevent thymic involution in response to the vesicant, nor involution of the spleen. The lymph nodes were less sensitive, and although they showed a suggestive reduction in weight in the adrenalectomised animals which had been injected with the vesicant, the reduction did not attain a statistically significant level.

A subsidiary object of this experiment was to determine whether a satisfactory prediction could be made of the behaviour and amount of the total lymphoid tissue from an analysis based on other more easily measured quantities. The quantities examined in this respect were the body weight and the weights of the superficial lymph nodes, thymus and spleen. The relation of each of these to the total lymphoid tissue was separately determined by calculating the residual (error) regression of the quantity in question on the total lymphoid tissue, after elimination of the effects of variation between subgroups. The calculations are not shown in extenso, but in table VI are set out the regression coefficients, with the corresponding values of Student's " t ".

It was found that the weights of the superficial lymph nodes and of the spleen were significantly correlated with that of the total lymphoid tissue, but that the regression coefficients for the other two quantities did not differ significantly from zero. It was then possible to calculate a regression equation for predicting the behaviour of the total lymphoid

tissue from knowledge of that of the superficial lymph nodes and spleen. This equation was :

$$Y = 1 \cdot 445 \, x_2 + 0 \cdot 680 \, x_4$$

where the symbols represent deviations from the means, x_2 being the deviation of the superficial lymph nodes and x_4 that of the spleen.

TABLE VI

Values of regression coefficients, with corresponding values of Student's "t",
in comparisons of individual measurements with the total lymphoid tissue

		Regression coefficient	"t"
Initial body weight (x_1) . .	Experiment 1	1·2140	1·633
	,, 2	0·3225	0·223
Superficial lymph nodes (x_2) .	,, 1	1·9625	5·167 *
	,, 2	1·853	6·07 *
Thymus (x_3)	,, 1	2·383	1·925
	,, 2	1·406	1·407
Spleen (x_4)	,, 1	0·8546	6·014 *
	,, 2	0·4455	3·98 *

* Value of "t" significant.

Experiment 2

In this experiment, the fact that the experiment was performed in three stages considerably complicated the analysis of variance by introducing a fourth main component, "stages", into the subgroups sum of squares, and by increasing the number of interactions to eleven. The subgroups in this experiment each consisted of 2 animals only.

As the animals were not completely randomised but were selected in pairs, it might be expected that the residual variance (within subgroups) would furnish too low an estimate of error. The analyses were accordingly also performed using the pooled high-order (2nd and 3rd) interactions as an estimate of error. It was found, however, that though the values of F were reduced by this procedure the significance of the result was not altered.

The initial body weights differed significantly between the three stages, and between the sexes. The animals in stage (b) (mean $21 \cdot 47 \pm 0 \cdot 26$ g.) were significantly heavier than those of stage (a) ($19 \cdot 91 \pm 0 \cdot 26$ g.) and stage (c) ($19 \cdot 52 \pm 0 \cdot 26$ g.). The females ($19 \cdot 71 \pm 0 \cdot 21$ g.) were lighter than the males of corresponding age ($20 \cdot 90 \pm 0 \cdot 21$ g.).

The superficial lymph nodes showed marked differences between stages, series and sexes, but not between treatments. The latter

difference, although suggestive, fell within the limits of sampling error. The difference between stages was due to the fact that the animals of stage (a), though the youngest, had heavier superficial lymph nodes (99·81±3·05 mg.) than those of stage (b) (79·44±3·05 mg.) and stage (c) (71·59±3·05 mg.). The difference between the two latter groups was not significant. In respect of the series difference, the superficial nodes in the anæsthetised (90·29±3·53 mg.) and adrenalectomised (95·79±3·53 mg.) animals showed no significant differences between themselves, but were heavier than the normals (79·88±3·53 mg.), while this group of nodes in the sham-operated animals (68·50±3·53 mg.) was significantly lighter than the normal. The marked depressant effect of sham-operation on the weight of the superficial nodes was probably due to stimulation of the adrenal cortex as a result of the operation.

The sex differences also were highly significant. In all four series, and in stages (a) and (b), the female superficial nodes were definitely lighter than the male. In stage (c) the difference fell just short of formal significance.

The thymus weight did not differ significantly between stages, but there were highly significant treatment and series effects, and moderately significant sex effects, the females having heavier thymuses than the males. Further analysis of the series difference showed that the mean thymic weight was significantly reduced by formalin in the normal, anæsthetised and sham-operated series, but was not affected in the adrenalectomised series, which in fact showed an apparent rise in thymic weight after injection of the drug.

The mean gross weight of the spleen differed significantly between stages:—stage (a), 207·32±9·2 mg. ; stage (b), 162·88±9·2 mg.; stage (c), 119·72±9·2 mg. The reduction in splenic weight as a result of treatment was moderately significant when taken over all four series. When the adrenalectomised animals were excluded from the comparison the significance of the difference was much increased, as there was no reduction—in fact a non-significant rise—in the gross splenic weight of the adrenalectomised animals as a result of the injection of formalin. The highly significant series difference was almost entirely due to the difference between the adrenalectomised series and the other three series taken together. The adrenalectomised animals had significantly larger spleens (212·04±10·67 mg.) than those of the other three series (normal, 148·75±10·67 mg. ; anæsthetised, 144·41±10·67 mg. ; sham-operated, 148·0±10·67 mg.), which did not differ significantly among themselves. The sex difference in gross splenic weight was also highly significant. This was due to the fact that the males had larger spleens than the females, especially in stage (b), and that the male spleen responded more markedly than the female spleen to the action of formalin.

The analysis of the total lymphoid tissue gave more definite results than that of the superficial lymph nodes. There were highly significant

differences between stages :—stage (a), $279 \cdot 53 \pm 8 \cdot 39$; stage (b). $259 \cdot 94 \pm 8 \cdot 39$: stage (c), $223 \cdot 35 \pm 8 \cdot 39$ mg. The reduction in lymphoid tissue as a result of treatment was also highly significant. In this respect, therefore, the analysis of a sample—the superficial lymph nodes—in which this difference was *not* significant, gave erroneous conclusions on the behaviour of the lymphoid tissue as a whole. The reduction in lymphoid tissue resulting from the injection of formalin was, however, confined to the three non-adrenalectomised series. In the adrenalectomised series there was a rise in total lymphoid tissue after injection ($310 \cdot 0 \pm 13 \cdot 7$ mg. as against $280 \cdot 67 \pm 13 \cdot 7$ mg.), a difference which falls just short of significance.

The series difference in total lymphoid tissue was also highly significant. Study of the series means showed that the difference between the normal ($246 \cdot 87 \pm 9 \cdot 7$ mg.) and anæsthetised ($254 \cdot 16 \pm 9 \cdot 7$ mg.) series was not significant, but that the sham-operated animals had considerably less ($220 \cdot 71 \pm 9 \cdot 7$ mg.) and the adrenalectomised animals considerably more ($295 \cdot 33 \pm 9 \cdot 7$ mg.) lymphoid tissue than the normal or anæsthetised animals. An indication that a series difference of this type was present had already been furnished by the analysis of the weights of the superficial lymph nodes.

The highly significant sex difference in total lymphoid tissue was ascribable to the circumstance that in all four series the males had more lymphoid tissue than the females. The males also showed a significant reduction in total lymphoid tissue after the injection of formalin (injected, $256 \cdot 37 \pm 9 \cdot 7$ mg. ; not injected, $301 \cdot 0 \pm 9 \cdot 7$ mg.) but the females did not (injected, $225 \cdot 33 \pm 9 \cdot 7$ mg. : not injected. $234 \cdot 38 \pm 9 \cdot 7$ mg.). This finding, which was foreshadowed by the analysis of the superficial lymph nodes, was in contrast to that of expt. 1, in which the females were more and not less susceptible to the action of the nitrogen mustard.

In this experiment. as in expt. 1, the relationship of the weights of the component organs of the lymphoid system to that of the total lymphoid tissue was examined by calculating the regressions of the body weight, superficial lymph nodes, thymus and spleen on the total lymphoid tissue. It was again found that only the weights of the superficial nodes and the spleen were significantly related to that of the total lymphoid tissue, the coefficients for the other two quantities not differing significantly from zero. In expt. 2 the regression equation for predicting the total lymphoid tissue from a knowledge of the weight of the superficial lymph nodes and spleen was found to be

$$Y = 1 \cdot 780 \, x_2 + 0 \cdot 427 \, x_4$$

where the symbols have the same meaning as in the equation obtained from the data of expt. 1.

The two regression equations do not differ greatly, and it would seem that for practical purposes an easily determined " index of lymphoid tissue " of the form $1 \cdot 5 \times$ superficial lymph nodes $+ 0 \cdot 5 \times$

spleen would allow a reasonably good prediction of the behaviour of the total lymphoid tissue in mice of this age and strain. Such an index would tend to prevent the drawing of erroneous inferences on the behaviour of the lymphoid tissues which might result from an analysis of a small sample only. Such an error would have occurred in expt. 2 if conclusions on the behaviour of the lymph nodes in response to the injection of formalin had been made from the analysis of the superficial lymph nodes only. Analysis of the behaviour of this index showed it to respond in a similar manner to the weight of the total lymphoid tissue in both experiments.

SUMMARY AND CONCLUSIONS

1. $\beta\beta'$ dichlorodiethylmethylamine hydrochloride (nitrogen mustard) differs from formalin in its effect on the weight of the lymphoid organs of mice. Adrenalectomy protects against the lymphoid involution induced by formalin but affords little protection against the involution induced by nitrogen mustard. Significant reduction in the weight of the thymus and spleen, and suggestive reduction in that of the total lymphoid tissue occurred in adrenalectomised animals treated with nitrogen mustard. The thymus was decidedly more sensitive than the spleen or lymph nodes to the action of nitrogen mustard and the effects of adrenalectomy.

2. Sham operation, without adrenalectomy, produced a considerable reduction in the lymphoid tissue.

3. Several sex differences were noted. The lymphoid organs of female mice were more susceptible to the action of nitrogen mustard than were those of male mice, but less sensitive to the action of formalin. Male mice had, in general, larger superficial lymph nodes, larger spleens and more total lymphoid tissue, but smaller thymuses, than females.

4. Considerable differences were found between the amount and response of the lymphoid tissue of batches of the same strain of mice, differing only slightly in age, when subjected to identical experimental procedures on successive occasions. This finding indicates that uncontrolled comparisons relating to the amount of lymphoid tissue are not always permissible between the results of successive experiments carried out on the same strain of animals, even when the ages and weights of the animals used in the different experiments are similar.

5. A technique is described for obtaining an estimate of the total amount of lymphoid tissue in the animal body. Study of the relationship of the body weight and the weights of the superficial lymph nodes, thymus and spleen to this estimate of the total lymphoid tissue showed that only the weights of the superficial lymph nodes and of the spleen were significantly correlated with that of the total lymphoid tissue. A regression equation was calculated for each

experiment, relating the total lymphoid tissue to the other two easily measured quantities. The two equations showed good mutual agreement.

6. It is suggested that such an " index of lymphoid tissue " might prove useful in future quantitative studies on the lymphoid tissue.

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SHORT ARTICLE

616 . 136 . 7—005 . 6 : 616 . 13—002 . 2

MURAL THROMBOSIS IN THE RENAL ARTERY AND ITS RELATION TO ATHEROSCLEROSIS

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(Plate CLV)

The thickening of the intima in atherosclerosis is made up of fat and fibrous tissue and sometimes also of calcium. Until recently it was assumed that the intimal fibrosis developed as a growth of connective tissue in response to the irritative effect of the fatty substances, but Duguid (1946, 1948) has reintroduced the idea that mural thrombosis may be the basis of atheroma and has shown that thrombi can frequently be demonstrated in thickened aortas. I can confirm the finding of Duguid that fibrinous deposits of various sizes and showing various degrees of organisation and incorporation into the intima can be demonstrated in about 35 per cent. of adult aortas showing atheroma. If these deposits are important in the genesis of the fibrous thickening, one would expect them to occur also in other arteries than the aorta, especially those in which atherosclerosis is common. I have made a detailed study of 50 pairs of renal arteries in search of these deposits and have taken sections from both the thickened and the apparently normal portions. The results show that thrombi occur frequently in the atherosclerotic parts of these arteries, that they are rare in the relatively unaffected parts and are absent from the normal parts.

Atherosclerosis first affects the origin of the renal artery and the changes are usually maximal in the first centimetre. In persons over 40 years of age it is exceptional to find no disease at this point. In the middle third, however, the disease is rarer, except at the orifices of branches where small plaques are often found.

Methods

The material was taken at autopsies performed on routine hospital and medico-legal cases, a dissection being made of the renal arteries and part of the related aorta. Sections were taken from the middle third of one artery and the thickened first part of the other. The first was fixed in the distended position by perfusing it with formol-saline at a pressure of 100 mm. of mercury and sections cut from a piece. 3 mm. in length, taken from the middle third. Distension reduced the folds in the internal elastic lamina and facilitated the study of the intima. The opposite vessel was fixed undistended and a piece was taken for section from the first centimetre. Distension was unnecessary here because atherosclerotic thickening reduces or prevents folding of the intima. Fifty sections each 15 μ thick were cut by the freezing method from both pieces and 9 sections chosen at intervals in the order of cutting, in order to obtain a fairly representative picture of this short length of vessel. Six sections were stained with Sudan III and hæmalum and 3 with orcein and hæmalum.

Histology of the deposits

After death, clotted blood may adhere to the intima. It usually appears in sections as a layer of homogeneous-looking plasma containing a variable number of red cells and leucocytes which are scattered through it or lie against the endothelium. Sometimes there is no plasma and only a few blood cells are seen in the crevices of the intima.

The appearance of these ante-mortem deposits alters with their age. What appears to be the first stage of mural thrombosis is seen in fig. 1. There is a thin network of fibrin on the surface enmeshing leucocytes and red cells. It appears to be firmly adherent to the intima but its free surface is ragged and not covered by endothelium.

Structures representing stages in the transformation of mural thrombi into the fibrous thickening of the intima seen in atherosclerosis are shown in the following figures. With organisation, endothelium grows over the thrombus and at the same time the fibrinous matrix becomes condensed. This is shown in fig. 2, which illustrates a thrombus of longer standing. It has a non-cellular component of granular material staining deeply with hæmalum, and a covering of endothelial cells which appear to have grown over the deposit so as to bind it to the intima. The granular material is composed of disintegrating red cells and condensing fibrin. Leucocytes are intermingled and seem to retain their staining properties for some time, though gradually the cytoplasm and finally the nuclei disappear. Fig. 3 shows another deposit containing red and white cells and covered by endothelium.

Deposits can occur repeatedly, one on top of another, giving a laminated appearance as shown in fig. 4. Although some damage has been done during the cutting of this section, the thrombus can be seen to be made up of three layers of different ages. The innermost layer consists of a fine network of fibrin containing numerous red cells and leucocytes; the middle layer has fewer red cells and the leucocytes have pyknotic nuclei; the outermost layer is devoid of red cells and shows only scanty nuclear remnants. The underlying intima is thickened by fibrous and fatty tissue. In fig. 5 a four-layered deposit is seen. The layers are composed of faintly staining homogeneous material separated by thin strata of red cells and a few globules of fat.

Later stages in the organisation of a thrombus are not convincingly demonstrable because the depth of staining of the ground substance is gradually lost and distinction between it and the fibrous tissue of the intima becomes increasingly difficult. In fig. 6 a small thin strip of lightly-staining material free of nuclei lies just beneath the endothelium. Perhaps this is a very late stage in the organisation of a deposit. The laminated appearance of the fibrous tissue frequently seen in greatly thickened intimas is again in keeping with the attempt to explain thickening on a mural thrombosis hypothesis.

Results

Only 8 of the 50 cases were below the age of 40 years; the rest were between 40 and 80. More males than females were examined, except in the 60-69 age group, where there were 7 of each sex.

Of 50 autopsy specimens, 19 (38 per cent.) showed ante-mortem deposits. Altogether 22 deposits were found, 17 in the thickened first part of the arteries and 5 in the thinner middle part. In 3 cases there were deposits in both regions and in one thickened part, 4 were seen. They occurred in both sexes in each decade from 40 to 80. None was found in the 8 cases below 40 years of age. No structure of possibly post-mortem nature was included in these figures. Deposits were found about three times as frequently in the first centimetre of the renal arteries as in the mid-zone, where 75 per cent. of the sections showed little or no atherosclerosis. There is thus at least a spatial connection between

MURAL THROMBOSIS IN THE RENAL ARTERY

FIG. 1.—A recent intimal deposit of fibrin in a renal artery enmeshing a few leucocytes. Sudan III and hæmalum.   × 230.

FIG. 2.—Part of an older deposit.   It is covered by endothelium and contains fat globules and degenerating red cells and leucocytes. Sudan III and hæmalum.   × 92.

FIG. 3.—A long thin deposit containing red cells and leucocytes and covered by endothelium. Orcein and hæmalum.   × 140.
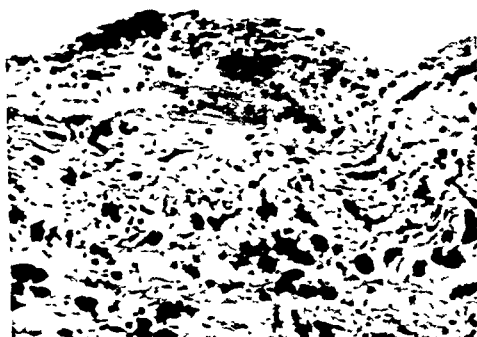
FIG. 4.—A three-layered deposit. Sudan III and hæmalum.   × 160.

FIG. 5.—A four-layered deposit with interspersed red cells and fat globules. Sudan III and hæmalum.   × 98.
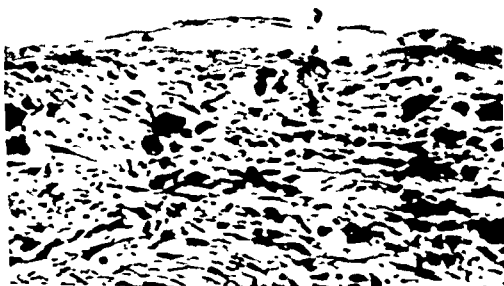
FIG. 6.—A later stage in the organisation of a mural thrombus. Sudan III and hæmalum. × 170.

atherosclerosis and deposits. There was no demonstrable connection between the finding of mural deposits in the renal artery and the cause of death. They were found in cases of sudden death, thus excluding the possibility of what may be called " terminal thrombosis " as a factor in the development of mural deposits. One case showed syphilitic disease of the renal arteries associated with mural thrombi.

Discussion

The occurrence of small mural thrombi in the main renal arteries does not appear to have been described previously. Duguid (1948) found deposits in 38 per cent. of his series of 50 aortas. As stated previously I have found them also in the same situation in about the same percentage of cases. Thrombi thus occur in approximately 38 per cent. of aortas, in 34 per cent. of first parts of renal arteries and in 10 per cent. of middle parts of renal arteries ; in the last position atherosclerotic changes are slight. This close association of thrombi and atherosclerosis is presented as further evidence in support of the hypothesis that mural thrombosis is an important factor in the development of atherosclerotic thickening of arteries.

The renal arteries of this series did not reveal any of the large deposits with invading capillaries which are sometimes seen in the aorta. Organisation is mainly a condensation process and is not, apparently, accompanied by the ingrowth of capillaries from either the covering endothelium or the underlying intima.

Summary

Small mural thrombi were found in relation to the intima in 19 out of 50 pairs of renal arteries, most of them being situated in the atherosclerotic first centimetre. They were less frequent in the less atheromatous middle third and were not seen at all in the normal parts of the vessels. It is suggested that this is further evidence in support of the hypothesis that mural thrombosis is an important factor in the development of atherosclerotic thickening of arteries.

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DUGUID, J. B. . . . . 1946. This *Journal*, lviii, 207.
,, ... 1948. This *Journal*, lx, 57.

BOOKS RECEIVED

Hæmolytic disease of the newborn

By MARGARET M. PICKLES. 1949. Oxford : Blackwell Scientific Publications. Pp. x and 181 ; 10 figs. on 6 plates and 15 text figs. 15s.

Dr Pickles has written a useful and comprehensive monograph on hæmolytic disease of the newborn which will be of value especially to pædiatricians and clinical pathologists called upon to cope with the practical problems involved. After an excellent historical introduction, the Rh antigens and antibodies are discussed, and both the Fisher-Race and Wiener notations are given. Dr Pickles wisely emphasises the predominant importance of antigen D in determining Rh positive or negative and the probable error in genotyping is well explained. The properties of Rh antibodies are lucidly expounded, yet the complexities of the subject are not underestimated, and the wealth of detail, given with commendable brevity, will assist the practising serologist.

Dr Pickles's experience in the correlation of maternal antibody titres with the severity of the hæmolytic disease is of great interest and her conclusion that low-titre antibodies acting over a long time are not less harmful than high-titre antibodies acting for brief periods accords with the reviewer's experience. In discussing the mechanism of immunisation, the great importance of transfusion in initiating the process is rightly stressed. Interesting examples are given of the variable antigenic power of cells apparently identical serologically and of the power of C and E antigens to provoke an anamnestic rise of anti-D. The pattern of the disease is well described and the importance of correct differential diagnosis in stillbirths emphasised, while in live births the indications for one or other form of treatment are given in detail and the dangers of early induction are not underestimated. We are glad to read Dr Pickles's firm statement of opinion that the importance of early treatment outweighs the desirability of delaying in order to achieve a more precise assessment of severity. It is interesting to observe that causes of kernicterus other than icterus gravis are gradually being discounted as more thorough investigations reveal Rh incompatibility in practically all cases, even those in which at first sight other circumstances might be held responsible.

The techniques of transfusion are clearly explained and Dr Pickles's modified method for exchange transfusion reads as if it should be both simple and satisfactory.

Throughout the text misprints are few, but on page 93 (line 7 from foot) surely " normoblasts " and " erythroblasts " should be transposed. On page 111 the intracorpuscular biliverdin should be stated in μg. as in the succeeding line. In the appendix there are a few unfortunate errors. In table XVI R_0r and CDe/cDe conflict : clearly $R_0r = cDe/cde$ is intended, and on page 162 $R'r$ is given as CDe/cde instead of Cde/cde. At the foot of page 158 a curiously inverted and misleading statement is made about D^u cells. Since D^u antigen is capable of stimulating anti-D in an Rh-negative recipient surely this should read " about half of these cell types contain a D^u component which may give *false negative reactions* by *failing* to react with some anti-D sera."

Hæmolytic disease of the newborn is not an easy subject on which to write lucidly and if there are occasional obscure paragraphs, on the whole they are remarkably few. We have no doubt that this book will be found of great value to practical workers, especially on the clinical side.

Heavy metal prosthetic groups and enzyme action

By Otto Warburg ; translated by Alexander Lawson. 1949. London : Oxford University Press (Geoffrey Cumberlege). Pp. xii and 230 ; 48 text figs. 18s.

The publication of a new book by Professor Otto Warburg is an important occasion for biochemists. As he says on p. 166, " The nature of the autoxidisable part of living matter, that is, the part which reacts with oxygen, has been since the time of Lavoisier the main problem of respiration," and it is almost exclusively this problem that he deals with in the present volume. The subject-matter is therefore rather more compact than the title would suggest. It is a field in which Professor Warburg's researches have been eminent for several decades. His approach is a truly biochemical one, with proper balance between the organic-chemical and biological sides. The treatment is historical, and amounts in effect to a partial scientific autobiography, emphasis being always on the work of his colleagues and himself and that of his scientific opponents. Indeed it may be thought that too much space is given to obsolete polemics, in which he was at cross-purposes in turn with H. Wieland, M. Dixon and D. Keilin.

Starting with the action of KCN, the charcoal model and the catalysis of oxidation by iron in general, the book proceeds, always with a wealth of experimental detail, to the quantitative study of the effect of CO on respiration. Here is given a full description of those classic experiments in which the absorption spectrum of the CO compound of the " Atmungsferment " was measured. Ultimately the position is reached in which Warburg's " oxygen-transporting iron " can be identified with cytochrome a_3. At every stage is found a valuable re-statement of the fundamental experiments on which all our present knowledge rests, but only very rarely does Professor Warburg indulge in speculation on possible future trends.

The book has more than its share of misprints and garbled sentences ; *e.g.* on p. 25 " respiration " occurs twice instead of " glycolysis," on p. 44 is found " activity of the metal " where some such phrase as " sensitivity of the method " is wanted, and on p. 141 the symbols for " light sensitivity " and " quantum intensity " are muddled. The translation is adequate, but exhibits a few odd mannerisms, such as the omission on every occasion but one of the hyphen from " oxygen transporting iron," and " the whole respiration proceeds over cytochrome."

The usefulness of the book would be enhanced by an alphabetical list of references and still more by an index.

How to befriend laboratory animals

By Charles W. Hume. 1949. London : The Universities' Federation for Animal Welfare. Pp. 16 and covers. 3d.

This pamphlet may be noticed here since its title is perhaps misleading. It is not, as might be supposed, an account of observations on animal behaviour or advice on how and how not to handle animals. It is part of " an attempt to break the deadlock, occasioned by the vivisection controversy, between humane scientists on one side and animal-protectionists on the other."

Biological actions of sex hormones

By HAROLD BURROWS. 2nd ed., 1949. Cambridge, at the University Press. Pp. xiii and 615. 42s.

Four years have passed since the first edition of this book was published and reviewed in this *Journal* (1946, lviii, 311). This, the second edition, has been entirely revised and re-set. An indication of the rate of growth of the subject is the fact that the list of references now occupies 36 pages instead of the former 24 and that the text occupies an additional 100 pages. The arrangement of the subject-matter is as before, gonatropin being considered first, then the gonadal hormones (androgens, œstrogens and progestins) and finally the sex hormones of the adrenal cortex. Those who have used this reference book in the last four years can testify to its great value and accuracy. The amount of information which it now contains is prodigious. The subject is one in which progress is constantly being made and therefore no book of this type can ever be said to present the last word. It is to be expected, however, that the great impetus given to the study of the biological actions of the sex hormones which followed their discovery in the '30s will now show some slowing down.

Handbook of bacteriology

By J. W. BIGGER. 6th ed., 1949. London : Baillière, Tindall and Cox. Pp. xv and 547 ; 24 figs. in colour on 4 plates and 109 text figs. 20s.

The fifth edition of this popular manual was published in 1939 and reprinted in 1945, 1946 and 1948. The sixth edition, which appeared in October 1949, is thus almost a new book. Two chapters have been added, eleven have been rewritten and all the rest have been extensively revised. The text has been increased from 455 to 535 pages plus an index of 12 pages. Nonetheless, Bigger's *Handbook of bacteriology* remains what it set out to be in 1925—a relatively short text primarily intended for students and non-specialists. The selection and arrangement of material are well made, the text is easily read and clear in meaning and the illustrations are excellent. The author feels it necessary to defend " a certain dogmatism in the presentation," but few will quarrel with this in a book specifically written for beginners, to whom it can be recommended with confidence. It may be permissible, however, to note that, in England and America at least, yellow fever vaccine is not now prepared from a neurotropic strain of virus but from strain 17D, a pantropic strain attenuated by growth in chick embryo from which the brain and cord have been removed.

Jordan-Burrows textbook of bacteriology

By WILLIAM BURROWS, with the collaboration of FRANCIS BYRON GORDON, RICHARD JANVIER PORTER and JAMES WILLIAM MOULDER. Fifteenth edition, 1949. Philadelphia and London : W. B. Saunders Company. Pp. xx and 981 ; 265 text figs. (2 in colour). 45s.

This is a good, sound book and a popular one, as may be judged by the appearance of fifteen editions in 41 years. Its scope and treatment make it eminently suitable for the serious and advanced student as well as for the laboratory worker who requires practical help with diagnostic problems. Probably it contains more than most elementary students will wish to read, and less than most research workers will demand of evidence and discussion ; but it remains one of the most useful and accurate texts within the compass of a single volume of reasonable size. Its range

is remarkable in that it deals not only with the usual pathogens and their diseases on standard lines, but with laboratory methods, bacterial physiology, water and sewage, milk and food, medical mycology, medical parasitology, and viruses. A well-balanced judgment is expressed on problems of bacterial nomenclature and the usages will be generally acceptable, particularly the retention of the genus Staphylococcus and the absence of any attempt to give specific names to the viruses. The illustrations are of a high standard and include many striking electron micrographs. It may certainly be recommended as a book that will contribute to the work of any laboratory.

Green's manual of pathology

Revised by H. W. C. VINES. 17th ed., 1949. London : Baillière, Tindall and Cox. Pp. viii and 1200 ; 19 figs. in colour on 12 plates and 730 text figs. 42s.

In these days of highly priced textbooks this attractively produced, well illustrated and easily read manual of pathology is, at two guineas, extremely good value for the money. It follows the general lines of its predecessors, the first part dealing with the principles of general pathology, the second with systemic pathology, especially in its relation to clinical medicine. The reproductions of gross specimens and the photomicrographs are almost without exception of a very high order. Nonetheless, the text is not without grounds for criticism, as is perhaps to be expected in any textbook written on general lines and covering such a wide field as pathology. The description of Paget's eczema is unsatisfactory and must leave the student in considerable doubt as to the real nature of the lesion. One wonders if " ulcero-cancer " (of the stomach) is a desirable term. Surely ulcer-cancer is better, or even *carcinoma ex ulcere.* And is it necessary to perpetuate the word " epithelioma " ? Squamous carcinoma is much to be preferred. In discussing status lymphaticus it would be well to explain that there is no pathological evidence of any relationship between an enlarged thymus and unexplained sudden death. The statement that the normal hæmoglobin content of the red cells is equivalent to 13·5 g. of hæmoglobin per 100 c.c. of blood seems unusual in view of the fact that the National Physical Laboratory accepts a figure of 14·8 g., and it is surely a novel conception that megaloblasts result from a failure of free multiplication of the hæmocytoblasts owing to the absence of thyroxin, vitamin C and the anti-anæmic principle. Most of the book, however, is beyond reproach and sections such as that dealing with diseases of the ductless glands, where the author is in his own field, are particularly good.

The 16th edition of this English classic, also revised and enlarged by Vines, was reviewed in this *Journal* in 1940 (li, 166).

INDEX OF SUBJECTS

Journal of
Pathology and Bacteriology

INDEX OF AUTHORS